Examining the Coexistence of Splachnaceae Mosses in Newfoundland Peatlands

by

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Abstract

Coexistence amongst highly similar species has captured the imagination of many ecologists. In my work I've endeavoured to answer the question of how *Splachnum ampullaceum* and *Splachnum pensylvanicum* coexist in peatlands in Newfoundland. I've employed individual based simulation modelling in conjunction with machine learning to answer if coexistence is facilitated most by dispersal differences or by competitive similarity. Experimental work examined dispersal differences and competitive ability directly. I found that coexistence is facilitated primarily by temporal niche separation via differing phenology, and substrate availability. My experimental work shows spore dispersal is dependent on distance to nearest moss population with no evidence of species-specific differences. The competitive ability of the two species was shown to vary according to moisture with potential facilitation effects at high population densities. Together the results show that the two Splachnaceae are more dissimilar than they appear, with niche separation along temporal and hydrological axes.

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Chapter 1: Grand Intro

This thesis was undertaken to understand coexistence, a fundamental component of biodiversity. How could evolution produce so many distinct species with competition acting to eliminate inferior species? Evolutionary constraints are a satisfying answer for why no one species could be perfectly adapted to all situations (Hoffmann 2014), however it doesn't satisfactorily explain how many seemingly similar species could coexist at the forest, field, or peatland scale. I was fortunate to fall into a lab exploring exactly that question, using a, in my opinion, immensely fascinating and tractable group of coprophilous mosses. The layout of this thesis is as follows: I first introduce the question in a bit more detail, along with the study species, then a manuscript chapter describing an individual-based simulation model I wrote to explore community dynamics and coexistence criteria, then an experimental chapter in which I attempt to understand dispersal and competition between two species of mosses, followed finally by a small conclusory synthesis with what I've learned from this project and how I've come to think about coexistence.

Communities often support many ecologically homologous species, a classic example being the so called “Paradox of the Plankton” in aquatic ecosystems (Hutchinson 1961). These species are striking examples of exceptions to the competitive displacement principle. The competitive displacement principle (*sensu* DeBach 1966), is an ecological tenet stating that no two species that share a niche completely can coexist in the same environment. These apparent exceptions to the competitive exclusion principle spawned a large body of research into what mechanisms allow for coexistence between highly similar species. Although less dramatic, systems as simple as two species coexisting despite appearing to share a niche are intriguing avenues for research, and some authors have argued that species poor systems warrant special attention when attempting to understand coexistence (Valladares et al. 2015).

An ecological niche is defined variously in the literature (Grinnell 1917; Elton 1927; Hutchinson 1944). For the purposes of this study, I will consider a niche as an "n-dimensional hypervolume" from Hutchinson (1944). This defines the niche to include each factor that influences population dynamics as an axis in a high-dimensional parameter space, although in practice niche space may, in certain cases, be summarized efficiently with many fewer axes. This is accomplished by assuming strong covariance amongst niche variables (Jackson et al. 2009). Without performing such dimensionality reduction, the axiom of inequality (Hardin 1960) applies, stating that no two species will exactly share a niche. Since no two species exactly share a niche, it is useful instead to explore what degree of niche overlap with respect to which niche axes can niches be shared among species without causing competitive displacement, i.e. permit coexistence as in the concept of limiting similarity (MacArthur and Levins 1967).

Several key niche axes have been identified as important for coexistence. Early classical work focused on direct competition, specifically resource partitioning (Gause 1932). Theory developed to include a dizzying array of mechanisms, broadly categorized as stabilizing or equalizing, depending on whether they increase or decrease species similarity, respectively (Chesson 2000). These mechanisms include, but are not limited to, heteromyopia (Murrell and Law 2002), storage effects and nonlinearity of competition (Chesson 2000), aggregation (Hartley and Shorrocks 2002), and spatial heterogeneity (Amarasekare 2003). A body of theory explaining coexistence by discounting the power of competitive displacement, neutral theory, arose in the early 2000's to explain the huge diversity of trees in tropical forests (Hubbell 2001). Neutral theory makes the assumption that all individuals are competitively and ecologically equivalent, such that changing an individual's species label does not impact its fate (Chesson and Rees 2007; Hubbell 2001). This view implies that all diversity can be explained by evolution and stochastic extinction

(Hubbell 2001). Although interesting as a null hypothesis, neutral theory is not particularly useful for elucidating mechanisms of coexistence acting within a community, instead being useful at an ecosystem scale, especially for predicting macroecological patterns such as species abundance distributions (Matthews and Whittaker 2014; Leibold and McPeek 2006). This is especially true when competitive differences have been observed *a priori* within a species assemblage, as is the case for the Splachnaceae mosses examined in this study (Marino 1991). This leaves the mechanisms promoting coexistence and the strength with which they act within many communities open for investigation.

Pairwise coexistence is often defined in ecological literature as a situation in which both species have a positive invasion growth rate, indicating the potential to rebound from arbitrarily low population densities, the mutual invasibility criterion (Turelli 1981), or it can be defined as the ability of two species to coexist for a set length of time, the upper limit being indefinite coexistence. With simulations models especially, and potentially epistemologically, studying unbounded time periods is challenging. The ability to predict the dynamics of any system, including communities and ecosystems, deteriorates with time due to concept drift; the process in which the parameters governing interactions change over time (Schlimmer and Granger 1986), or due to chaos from a more mathematical standpoint (Hastings et al. 1993). For this thesis, species capable of coexisting for greater than 1000 years – 333 generations for these mosses – will be considered as indefinite coexistence.

Because the time-scale in which processes influencing coexistence operate can be on the order of decades and centuries, field experiments are, in and of themselves, often inadequate for exploring the mechanisms promoting coexistence. Field experiments when combined with modelling provide a means to explore potential mechanisms promoting coexistence and the

interactions among those mechanisms. Ideally, such an approach works best for a community or ecosystem for which there is a strong pre-existing domain knowledge, the dynamics are mathematically tractable, and the organisms and their environment are experimentally tractable.

In this study I have focused on a two species of Splachnaceae mosses, *Splachnum ampullaceum* Hedw. and *Splachnum pensylvanicum* (Brid.) Grout ex H.A. Crum., to examine the details of pairwise coexistence. These mosses both grow in peatlands on the island of Newfoundland, Canada. These species of mosses, as well as approximately half the species of this family, are coprophilous meaning they are limited to growing, primarily, on dung and also on carrion. In Newfoundland, they are most commonly found on summer moose (*Alces alces* L.) dung, as opposed to the pellet winter dung, and occasionally on the remains of animal carcasses; bones for example. Both species are found growing on the dung of other large herbivores (Marino personal observations), but this has never been observed in Newfoundland. Additionally both species of moss are entomophilous, more precisely myophilous, meaning they have their spores dispersed by flies (Diptera) (Bryhn 1897; Bequaert 1921). To facilitate entomophily, the mosses use a complex set of odour and visual cues to attract fly visitors (Marino et al. 2009; Marino and Raguso 2016). Fly visitors pick up small clumps of adhesive spores and disperse them to dung.

Splachnum ampullaceum is a circumboreal species with relatively tall sporophytes (setae 15 - 65 mm). The inflated hypophysis (the part of the seta just below the capsule) ranges in colour from yellow to pink forming a large visual display when present in high numbers. Volatile production in *Splachnum ampullaceum* is concentrated in the swollen hypophysis and the volatiles identified from sporophytes included short chain oxygenated compounds, unsaturated irregular terpenoids, fatty acid-derived 6- and 8-carbon alcohols and ketones, the aromatic compounds acetophenone and p-cresol and a group of 10 compounds suggesting esters of cyclohexanecarboxylic acid

(McCuaig et al. 2014). In contrast, *Splachnum pensylvanicum*'s distribution is restricted to eastern North America from Florida to Newfoundland, and has small green/red sporophytes (setae 4 - 9 mm) producing a relatively limited visual display. Volatiles produced by *S. pensylvanicum* include many of the same compounds emitted by sporophytes of *S. ampullaceum*, but lack the cyclohexane carboxylic acids and instead produce small amounts of compounds such as dimethyl disulfide and indole, compounds generally associated with carrion mimicking species (Jürgens et al. 2013).

In our study site in Newfoundland, the two species can be found growing in pure and mixed populations. A population in this case being defined as mosses capable of interbreeding; i.e. on the same dung pat. Each species of moss attracts a relatively distinct fauna of flies (Marino et al. 2009; Marino and Raguso 2016). This unique compliment of ecological dynamics make them a fascinating system for exploring coexistence. When found growing together within a peatland, they are an ideal model system for combining modelling and experimental research. Their biology has been well studied (Marino et al. 2009; Marino and Raguso 2016), they have a two to three year life cycle with well-defined life stage transitions. They are also amenable to *in situ*, transplantation, and *in vitro* growth experiments, and so I have endeavored to experimentally determine their vital rates, examine their growth and dispersal, and attempt to simulate their interaction in order to better understand how such similar species can coexist.

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Co-Authorship Statement

Both manuscripts presented here were authored with the help of Dr. Paul Marino. I was responsible for all aspects of the study design, execution, analysis, and manuscript preparation.

Chapter 2: Modelling the coexistence of *Splachnum ampullaceum* and *Splachnum pensylvanicum* in Newfoundland Peatlands

Abstract

In this study we examined coexistence between *Splachum ampullaceum* and *S. pensylvanicum* two coprophilous mosses in the family Splachnaceae coexisting in peatlands on the island of Newfoundland, Canada. We constructed an individual-based simulation model based on within patch Lotka-Volterra competition and varying timing and efficacy of spore dispersal. We subject model outcomes to sensitivity analysis in order to determine which model parameters are most influential in determining whether the two mosses would coexist for the duration of a 1000 year simulation. The sensitivity analysis was conducted by training 10 replicate deep neural networks and random forests on 5000 simulations with latin hypercube sampled parameter combination and examining variable importance. As expected growth rates were the most important parameter for predicting coexistence, followed by substrate availability and dispersal phenology. Additionally, species aggregation was shown to be a very powerful summary statistic for examining patch related coexistence.

Intro

Coexistence between ecologically homologous species is a frequent observation in nature that has fascinated ecologists. The competitive exclusion principle (Hardin 1960) has served as a useful axiom for defining the subtleties that differentiate species' niche space and promote biodiversity. The mechanisms that permit highly similar species to side-step the competitive exclusion principle, termed coexistence mechanisms, are numerous (see: Chesson 2000; Amarasekare 2003). Key mechanisms include differences in phenology (Fargione and Tilman

2005; Godoy and Levine 2013), dispersal, typically in the sense of a competition-dispersal trade-off (Tilman 1994), and resource patchiness (Hartley and Shorrocks 2002). It is worth noting that there exists a division of ecology focusing on the diversity of species assemblages wherein all competitors are considered ecologically equivalent, neutral theory. Neutral theory, though not strictly concerned with coexistence (one prediction of neutral theory is that coexistence is, in a sense, impossible; Hubbell 2005) is useful for making predictions about the long term behavior of ecosystems whereas niche theory is more useful for examining pairwise species relationships (Leibold and McPeek 2006). For the duration of this article only the niche-based theories of coexistence will be considered. A key conclusions of the literature regarding coexistence mechanisms are that species need to be sufficiently distinct to coexist (Chesson 1991) and that species may achieve coexistence under a variety of different frameworks, many of which include only one major mechanism (e.g. Fader and Juliano 2012). Since coexistence may be achieved a number of different ways, for a given system it is worth attempting to determine how its species coexist.

This study focuses on a two species metacommunity of mosses in the family Splachnaceae; *Splachnum ampullaceum* Hedw. and *Splachnum pensylvanicum* (Brid.) Grout ex H.A. Crum. These two mosses coexist in peatlands in Newfoundland, Canada competing for space on moose dung. Approximately half of the 74 species of Splachnaceae are coprophilous (Goffinet *et al.* 2004), growing only on dung and carrion. In order to facilitate dispersal to substrates that are limited in both space and time, coprophilous Splachnaceae employ flies (Diptera) to move spores to new patches, a condition called entomophily (Marino *et al.* 2009). These mosses have elaborate sporophytes to attract flies with a suite of visual and olfactory signals (Marino *et al.* 2009). Flies that interact with the sporophytes may pick up small clumps of adhesive spores.

Some proportion of those flies will transport spores to new substrate, in a manner analogous to pollination. This unique metacommunity is essentially a plant analog of the carrion fly communities of early aggregation models (Hanski 1981; Atkinson and Shorrocks 1981; Ives 1988) where resource patchiness and ephemerality can permit coexistence. These models allow coexistence when intraspecific competition inhibits the superior competitor more than its subordinate species.

Resource patchiness, as exemplified by resources such as carrion (Ives 1991), dung (Hutton and Giller 2004), and mosquito breeding pools (Fader and Juliano 2012), is frequently invoked as a mechanism by which species, particularly arthropods, coexist. Patchy and ephemeral resources support a diverse suite of organisms, with many more species than would be predicted from traditional competition theory (Atkinson and Shorrocks 1981). These ephemeral resource patches have been advocated as an excellent model to study ecological processes (Finn 2001; Barton *et al*. 2013). In the case of *Splachnaceae*, the ephemerality of the dung pat is not a consumptive process but instead a dispersal limitation. Several days after deposition the dung no longer attracts flies due to decreased odour output over time, after odour decays below a threshold the pat is closed to spore arrival. This system also differs substantially from typical angiosperm plant models common to coexistence research due to the biology of Splachnaceae (and bryophytes in general) such as their having no true roots, their lack of vertical growth, and their filamentous (protonematal) growth phase. Resources patchiness and emphemerality impose a dispersal dependence on coexistence, adding spatial and temporal dimensions to the question of coexistence, heightening the importance of dispersal.

Dispersal is a key component to the natural history of organisms bound to patchy and ephemeral resources and, as such, it is important to look at the degree to which varying the timing

(phenology), and magnitude of dispersal (number of effectively dispersing spores) influences the success of populations. A mechanism by which dispersal differences can act to slow or eliminate competitive exclusion in patch systems is intraspecific aggregation (Shorrocks *et al*. 1979). Intraspecific aggregation is the degree to which members of the same species cluster together in available habitat relative to a uniform distribution, this heightens intraspecific competition and reduces the growth rates of superior competitors to a greater degree than subordinate species, allowing coexistence when the inferior competitor persists in low density patches (Hartley and Shorrocks 2002). The model described in chapter one builds on the ideas presented by Marino (1991) where he shows with a simulation model that varying the degree of conspecific spore aggregation (as governed by a negative binomial aggregation distribution) allows coexistence between *Splachnum ampullaceum* and *Splachnum luteum* in peatlands in Alberta. Although no mechanism for how intraspecific spore aggregation occurs was given by Marino, the action of variable dispersal in a heterogeneous environment can lead to aggregation. It is likely through a combination of factors including: variable timing in the maturation of sporophytes growing on different patches, the variable time and location in which new, fresh patches of moose dung are deposited, and the short 2-3 day window in which new dung is attractive to flies, that spores of conspecifics become aggregated. If this is the case, the ecological traits of both moss species should show evidence of differentiation in dispersal strategies either by producing spores at different times and/or by varying the magnitude of dispersal. Phenological staggering is one mechanism by which spores of both species might become aggregated. Work by Nakazawa and Doi (2012) has shown that changing the degree of phenological overlap in a tri-trophic ecosystem model with sinusoidally oscillating phenologies can impact competitive hierarchies and allow coexistence. Recent work by Revilla *et al.* (2014) shows that coexistence can occur in

a two species community module given both species are staggered with respect to their shared resource. These patterns of dispersal differences permitting coexistence are a strong indication that dispersal difference could be driving coexistence between these Splachnaceae mosses.

In this paper, we seek to elucidate by means of individual based simulation modelling the role that the timing and magnitude of targeted dispersal play in modulating the effects of competitive asymmetry between *Splachnum ampullaceum* and *S. pensylvanicum.* We attempt to identify the strength with which both species attract vectors, the different phenological strategies the mosses employ to optimize competitive outcomes, and the degree of competitive asymmetry between them (explored further in a forthcoming paper by Hammill and Marino 2016). Additionally we aimed to elucidate the degree to which intraspecific aggregation can explain the likelihood of coexistence between the two mosses. To analyze coexistence in non-equilibrial individual based models, an approach centering on sensitivity analysis by means of analyzing variable importance was used (Gedeon 1997; Louppe et al. 2013). Variable importances were determined by machine learning techniques (random forests and deep artificial neural networks); these importance measures were used to identify the most influential from a suite of candidate predictors such as competitive ability (growth rate), attractiveness, and degree of aggregation. Competitive differences and dispersal magnitude (via attractiveness) were hypothesized to be the most important drivers of coexistence outcomes as they are the ecological forces driving competition. Phenology and substrate availability were hypothesized to play subsidiary roles in driving coexistence outcomes as these two forces modulate the degree to which the two species overlap in space and time. It was hypothesized that coexistence would only be possible when competitors are highly similar, because a benefit in terms of growth or dispersal should allow the dominant species to exclude the inferior species. Specifically, coexistence is expected when competitive

and attractiveness differences are minimal, with chances of coexistence negatively correlated with the magnitude of difference. Increasing phenological staggering and increasing substrate availability were hypothesized to improve the chances of coexistence in accordance with the phenology literature (Fargione and Tilman 2005; Nakazawa and Doi 2012; Godoy and Levine 2013; Revilla et al. 2014) and the habitat amount hypothesis (Fahrig 2013). Additionally we hypothesized that aggregation, specifically intraspecific aggregation is the key driver of dispersal mediated competitor stabilization (Marino 1991; Hartley and Shorrocks 2002).

Methods

Ecology of *Splachnum ampullaceum* and *Splachnum pensylvanicum*:

Splachnum ampullaceum is a circumboreal species with relatively tall sporophytes (setae 15 - 65 mm). The inflated ampulla-shaped hypophysis (the part of the seta just below the capsule) ranges in colour from yellow to red (at senescence) forming a large visual display when present in high numbers. Volatile production in *S. ampullaceum* is concentrated in the swollen hypophysis and the volatiles identified from sporophytes included short chain oxygenated compounds, unsaturated irregular terpenoids, fatty acid-derived 6- and 8-carbon alcohols and ketones, the aromatic compounds acetophenone and p-cresol and a group of 10 compounds suggesting esters of cyclohexane-carboxylic acid (McCuaig et al. 2015). In contrast, *S. pensylvanicum*'s distribution in North-America is east of the Appalachian Mountains from Florida to Newfoundland. *Splachnum pensylvanicum* has small green/red sporophytes (setae 4 - 9 mm) producing a reduced visual display. Volatile production is also concentrated in the hypophysis but *S. pensylvanicum* produces a different complex of volatiles, lacking cyclohexane carboxylic acids but producing dimethyl disulfide and indole which are known to attract carrion flies (Marino and Raguso 2016; Jürgens et al. 2013).

In our study site in Newfoundland, the two moss species can be found growing in pure and mixed populations (a population here being defined as mosses capable of interbreeding; i.e. on the same dung pat). Each species of moss growing alone attracts a different fauna of flies whereas the fauna of flies associated with mixed species populations does not differ from that found on either species growing alone (Marino and Raguso 2016). Both mosses have no herbivores, although slugs have occasionally been observed grazing on sporophytes (Marino and Coates personal observations). Populations take approximately two growing seasons to mature: spores are deposited and germinate the first year, undergo competition for space on the dung pat the second year, and typically produce sporophytes the third, followed by senescence and/or overgrowth by the surrounding *Sphagnum* mosses (Marino personal observations). This life history strategy facilitates stage-structuring and delimiting processes occurring at each stage.

Empirical Parameter Estimates:

We estimated the spore yield of each species by counting the number of sporophytes per cm^2 on two pure populations of each species, removed from the field and brought to the lab for counting. The two populations were split into a total of five approximately 10 cm^2 regions; the five replicates were averaged for each species. The number of spores produced per sporophyte was determined by immersing ten sporophytes of each species in one mL of water, vortexing the solution and estimating spore numbers using a haemocytometer. The product of sporophytes per cm^2 and spores per sporophyte gives the area specific yield for each species.

We estimated the relative attractiveness of both species from a fly trapping data set (Marino unpublished) in which flies were trapped on both species of moss as well as fresh dung during 20 days in July 2007. The number of flies trapped on pure populations of both species was standardized as a proportion of the number of flies trapped on fresh dung (age zero).

Modelling Ideology:

To examine the interplay of dispersal magnitude, phenology, competitive ability, and substrate availability in permitting coexistence, we developed an individual-based, also known as agent-based, simulation model. In the model, spore dispersal and competition were modelled in a spatially explicit framework; we sought to closely emulate the real world processes that shape this metacommunity. In order to stay rooted in the reality of these organisms, field and lab-based estimates of key parameters were used to inform the regions of parameter space examined. Parameters that weren't informed by experiment were estimated either from theory or domain knowledge of the two species and Splachnaceae in general (Marino *et al.* 2009).

Most coexistence theory is concerned with the mutual invasability criterion (Turelli 1981; Chesson 2000), a tenet stating that a community of species may coexist indefinitely provided all species have a positive per capita growth rate at arbitrarily low population levels. This work is instead concerned with persistence, the ability of a species to maintain greater than a minimum viable density for a given period of time (Caswell 1978). By relaxing the definition of coexistence to include sufficiently long persistence we acknowledge that competitive hierarchies, environmental dynamics, and many of the other implicitly static variables in coexistence theory may be subject to change, and that the ability of any model to predict dynamics deteriorates with time due to parameter drift, stochasticity, and chaos (Hastings et al. 1993).

The simulation model was written in Java and tracked the population dynamics within a simulated peatland. All subsequent analyses of model output were carried out using R (R Core Team 2014).

Model Overview:

The model simulates dung pats at several stages of moss colonization: recently deposited dung pats, immature populations, and mature (sporophyte bearing) populations. Each individual dung pat progressed through the three life-stages with transitions occurring between year steps. Colonized dung become immature populations and immature populations become spore-bearing populations in the next year. This stage-structuring is appropriate for modelling both *Splachnum* species of interest due to their approximately biennial life-cycle (Marino personal observations). Within years, two isolated major processes occur: competition and dispersal. Dispersal is the transfer of spores from mature populations to new dung; the dung is constrained to only receive spores for a brief window in which the dung is still odorous enough to attract flies (see "relative rate of spore transfer" below for more details). Spores of mature populations are dispersed to dung; this sets the initial conditions for competition in the next time step. Competition in this model is treated as a discrete time scramble for space between the two species. Protonemata, the filamentous immature stage common to mosses, compete for space to determine the species composition of the mature populations in the next time step. Census of proportional occupancy of the peatland by mature individuals of the two species occurs at the end of each year step (after competition and spore dispersal but before stage transitions occur).

Spatial Considerations:

The simulated peatland was circular with the locations of individuals given by a distance (l) from the centre and a counter clockwise angle (θ). A fixed number of dung pats were deposited throughout the simulation on random days. Each new dung pat was given a random pair of spatial coordinates with distance randomly distributed between 0 and the radius of the peatland, and θ randomly distributed between 0 and 2π radians. Randomly generated locations allowed for

stochastic spatial behaviour, it is important to note that this is not truly a uniform spatial distribution, as pats will be concentrated toward the center of the peatland, we feel that this no less realistic than a fully uniform distribution.

Space Competition

Within gametophyte populations, *Splachnum ampullaceum* and *S. pensylvanicum* protonemata underwent discrete time Lotka-Volterra competition for space. For modelling purposes, the dung pats were treated as being equivalently sized, accommodating 100 spatial units of coverage total for both species. The areal coverage for each species at each time step (one day) is calculated with difference equations 1 and 2,

$$\mathrm{N}_1(t+1) = N_1(t) + r_1 N_1(t)\left(1 - \frac{N_1(t)+\alpha_{12}N_2(t)}{k_1}\right) \quad (1)$$

$$\mathrm{N}_2(t+1) = N_2(t) + r_2 N_2(t)\left(1 - \frac{N_2(t)+\alpha_{21}N_1(t)}{k_2}\right) \quad (2)$$

where *N* represents the areal occupancy of *S. ampullaceum* or *S. pensylvanicum* (subscripts 1 and 2 respectively), k_1 and k_2 represent the areal carrying capacity for each species, r_1 and r_2 represents the daily areal growth rates, and α_{ij} represents the competitive pressure exerted by one unit coverage of species *j* on species *i* towards the carrying capacity of species *i*, and *t* represents the time in days since the beginning of the current growth year. Initial values for areal coverage are proportional to the number of deposited spores from the previous year.

There are key differences between this formulation of Lotka-Volterra competition and the standard competition equations. First, since the model works in units of areal coverage, as opposed to number of individuals, the carrying capacities are fixed by the amount of space on the dung pat. For simplicity, we can define the units of areal coverage (size) for both species to be

equal. Since the size of a shared dung pat is equal from the perspective of both species and their units of areal coverage are identical, their carrying capacities must also be the same. Then, we constrain the model such that the only barrier to growth for both species is the current occupation of space, the competitive coefficients for the two species must also both be equal to one. In this highly specific case, the only way in which competitive differences between species manifests is through *per capita* growth rate, as opposed to depending solely on competitive coefficients. A side effect of this formulation is that competitive exclusion within a dung pat at reasonable growth rates, given that both species dispersed to it successfully and that the sum of both species at time zero does not exceed the carrying capacity, is impossible. Competitive exclusion occurs in this model only when the inferior competitor is kept at a sufficiently low density that it fails to disperse successfully to new dung pats.

Spore Production and Transfer:

Mature populations disperse spores to dung at each day step. The magnitude of spore transfer between a given moss population and a dung pat is contingent on many factors. Firstly, dispersal depends on the spore output of the moss population. This depend on the coverage of both species, and their species specific parameters for phenology and areal yield:

$$P_{mj} = N_{mj} Y_m f_m(t) \quad (3)$$

Where P_{mj} is the units of areal coverage produced by moss species m in population j, N_{mj} is the coverage, Y_m is the species specific yield, and $f_m(t)$ is the species specific phenology discussed in more detail below.

The effective dispersal of a moss population further depends on the distance between the moss and the dung, as well as a species specific attractiveness modifier for the dung pat.

$$D_{mij} = P_{mj} R_{mi} k_e(d_{ij}) \tag{4}$$

Where D_{mij} is the number of units of areal coverage contributed by moss species *m* from population *j* to dung pat *i*, P_{mj} is the production calculated in equation 3, R_{mi} is the attractiveness modifier between moss species *m* and dung pat *i* (discussed in detail in section Relative Spore Transfer Rate) and $k_e(d_{ij})$ is an exponential dispersal kernel evaluated at the distance between the moss and the dung.

$$\mathrm{k}_e(d_{ij}) = e^{-\frac{d_{ij}}{b}} \tag{5}$$

Where *b* is a tuning parameter that sets the scale of dispersal that can be interpreted as the distance required to lose approximately 2/3 of the dispersal potential. The parameter *b* was set to 30 m for all simulations.

The total number of units of areal coverage of both species that arrive on a dung pat was kept as a running total of the inputs from all mature mosses for each day of the dispersal window.

$$S_{mi}(t+1) = S_{mi}(t) + \sum D_{mij} \tag{6}$$

Where $S_{mi}(t)$ is the time indexed quantity of units of areal coverage for species *m* on dung pat *i*. The time-step for equation six is one day. For each dung pat, S_{mi} at the end of the growing season was used as the starting point for competition in the following year step. All spores arriving on the dung pat are treated as arriving at the same time and germination difference were not considered.

Relative spore transfer rate:

To understand how the model treats spore transfer it is important to start with the observation that dung is not constantly attractive throughout its existence. Freshly deposited dung is more attractive to flies than at any subsequent time point. Flies cease visiting dung after approximately three days (Marino personal observations) and so the mosses have a very limited window in which to reach fresh dung. This curtailed window adds ephemerality to this patch system, contrasting with the consumptive process of the carrion fly models (Atkinson and Shorrocks 1981; Ives 1991). Because of the brevity of this window, all spores were treated as arriving at the pat at the same time, priority effects were not considered.

In this model, the decay in attractiveness of the dung was modeled as a gaussian function of time since deposition, the usual location/shape parameters μ and σ represent the time at which the dung is maximally attractive, and the time it takes to reach approximately a quarter of its original attractiveness respectively. Relative spore transfer was in turn calculated as a function of the attractiveness of each moss species' attractiveness and the attractiveness of the dung:

$$\mathrm{R}_{mi} = max\left(A_m \left| \frac{\mathcal{N}(x_i,\mu,\sigma)}{\mathcal{N}(\mu,\mu,\sigma)} - A_m \right|, 0\right) \quad (7)$$

R_{mi} is a modifier for spore transfer ranging between zero and one. Where A_m is the attractiveness of moss species *m* as a proportion of the maximal dung attractiveness also ranging between zero and one. $N(x_i,\mu,\sigma)$ is a gaussian decay function for the attractiveness of any dung pat *i* at age *x*. The attractiveness of the dung is scaled to range between zero (old and completely unattractive) and one (freshly deposited and maximally attractive). All dung pats were considered equally attractive; we feel this is a reasonable assumption due to the diversity of fly vectors averaging the impact of changes in individual odour compounds particular to the dung. The attractiveness of a

moss species is defined as a proportion of the maximum dung attractiveness. The rate of spore transfer between a moss population and a target dung pat depends on the difference in their attractiveness. When a dung pat is much more attractive than the moss (when a dung pat is fresh) spores move quickly from moss to dung, whereas, when a dung pat ceases being more attractive than the moss, spores stop moving to the dung pat. The rate of spore transfer also depends on the number of flies each species of moss attracts, which is proportional to the attractiveness of the moss.

Phenology:

The temporal differentiation between *Splachnum ampullaceum* and *S. pensylvanicum* with respect to when the populations of each species mature is important to consider when examining their coexistence. To account for differences in the timing of spore dispersal, the spore yield was modified by a sinusoidal function (Abrams 2004; Nakazawa and Doi 2012) dependent on the time of year.

$$f_m(t) = \frac{cos\left(\frac{2\pi}{T}(t-v_m)\right)+1}{2} \quad (8)$$

This modifier represents the proportion of the maximal daily specific areal spore yield that a species produces at a given time in the growing season. The function chosen was scaled to oscillate between 0 and 1 completing one full oscillation per growing season (T days). Different horizontal translations (v_m) were applied to the function to create varying degrees of phenological staggering between competitors (Appendix 1 Fig. S1). A uniform function was included to model dynamics in the absence of distinct dispersal phenology. Cosine with no translation is most similar to the observed phenology of *S. pensylvanicum*, with dispersal maxima early and late in the growing season. Cosine with a large horizontal translation (~75 days) was most

similar to the phenology of *S. ampullaceum* with one large maximum in the middle of the growing season. For convenience, translations of 37.5 days and 75 days will henceforth be referred to as sine and negative cosine. Given the approximately cosine and negative cosine phenologies of *S. ampullaceum* and *S. pensylvanicum* respectively, the observed phenological stagger of the two species is approximately 75 days.

Parameter Influence on Coexistence:

To examine the relative importance of attractiveness, competition, phenology, and substrate availability in allowing coexistence, parameter space was explored using latin hypercube sampling, where the distribution of each parameter is partitioned into equiprobable bins equal to the number of samples to be drawn (McKay et al. 1979). The growth rates were sampled from a uniform distribution between 0.01 and 0.10 (% day^{-1}), relative attractiveness was sampled from a uniform distribution between 0 and 100%, the number of new dung pats available per year was sampled from a uniform distribution between 2 and 75 (to capture a range of potential substrate availabilities). Phenology for both species was randomly sampled from four potential phenology functions, uniform, cosine, sine, and negative cosine. Non varied parameters were held fixed at the levels shown in table 1. All simulations consisted of two competitors, each a hypothetical moss playing the role of either *Splachnum ampullaceum* or *S. pensylvanicum*.

The status of every population was recorded at the end of each simulated year and the average percent coverage was calculated each year for both species. The absolute difference between the two is a measure of competitive outcomes. The time until first extinction is the first year that either of the two species had a mean coverage of less than 0.01 across the simulated peatland.

Influence of Conspecific Aggregation:

To examine the degree to which conspecific aggregation occurs through the action of dispersal differences, Ives' J and C statistics (Ives 1991; Fader and Juliano 2012) were calculated for the spore allotment on each dung pat at the end of a growing season and averaged for the peatland. Only the first and second year of each simulation were considered, as these metrics are most informative prior to the action of competition which acts to increase the interspecific aggregation of the competitive dominant and decrease the aggregation of the competitively subordinate species (in a two species system such as this). *J* represents the degree of enrichment relative to random allocation of individuals (or in this case units of coverage) in the number of conspecific individuals a member of species m encounters and is defined as follows:

$$J_m = \frac{\sum_i^n N_{mi}^2 - N_{mi}}{n\overline{N_m}^2} - 1 \quad (9)$$

where N_{mi} is the coverage of species *m* in population *i*, $\overline{N_m}$ is the mean coverage of species *m* in all populations, and $\overline{N_m}$ is the total coverage of species *m* for all populations. The *C* statistic represents the degree of covariance between the two species, and hence, mean interspecific aggregation. The *C* statistic is defined as follows:

$$C_{ap} = \frac{\sum_i N_{ai} N_{pi}}{N_{at}\overline{N_p}} - 1 \quad (10)$$

where N_a and N_p are the coverage of the two species on dung pat *i*, N_{at} is the total coverage of *S. ampullaceum* and N_p is the mean coverage of *S. pensylvanicum*.

An additional measure of interspecific aggregation, the Shannon-Weiner diversity was also used to measure aggregation. This statistic captures aggregation as decreases in the entropy of the species distribution.

$$S = -\frac{1}{n}\sum_{i}^{n} P_{ai}\ln P_{ai} + P_{pi}\ln P_{pi} \tag{11}$$

Here the diversity is averaged over *n* dung pats, with P_a and P_p are the proportional coverages of the two moss species, indexed by dung pat. *S* can range from approximately 0.7, where both species occupy one half of each dung pat respectively, to 0 where one species is absent from all patches.

Aggregation as treated in this paper is the result of dispersal differences, competition also has an effect on the evenness of species distribution amongst patches. To isolate the effects of dispersal mediated aggregation these metrics were only calculated at the end of the first and second year of each simulation, before any competition has occurred between the two species. The first and second year aggregation measures were averaged for each simulation.

In addition to *J*, *C*, and *S* statistics, several higher order aggregation statistics were included in the analysis. Sevenster's *T* statistic (1996) and Shorrocks and Sevenster's (1995) A statistic were examined and are defined as follows:

$$T_{ap} = \frac{1 + C_{ap}}{1 + J_p} \tag{12}$$

$$A = \frac{(1 + J_a)(1 + J_p)}{(1 + C_{ap})^2} \tag{13}$$

where T_{ap} represents the effect of aggregation of *S. pensylvanicum* on *S. ampullaceum*, $T_{ap} < 1$ is taken as sufficient for persistence of *S. ampullaceum* (Sevenster 1996) the complimentary statistic for *S. pensylvanicum* was also considered. Shorrocks and Sevenster's *A* statistic measures the relative strength of intraspecific aggregation relative to interspecific aggregation and is the product of the inverse of the two *T* statistics.

This suite of aggregation statistics were included and compared with the simulation parameters in determining the major influences on coexistence outcomes.

Determining Predictors of Model Outcomes:

In order to determine which features of a simulation were most influential in determining coexistence outcomes, we used a combination of two machine learning techniques to generate predictive models of simulation outcomes. Random forest (Breiman 2001) and deep neural networks (Schmidhuber 2015; LeCun et al 2015) were trained to predict the presence or absence of coexistence. To train the models, the simulation results for each of the 5000 latin hypercube parameter samples were collated with their respective parameters and aggregation statistics, some alternative codings of parameters were also included. This data set was split into 10 different random allotments of 2/3rds training set and 1/3rd test set; the column order was permuted to reduce the chances of variable ordering effects on model outcomes. Both a random forest and a deep neural network were trained on all ten training-sets. The prediction accuracy and area under their receiver-operator characteristic curves (AUC) were calculated from predictions made on the matching test-set. Both types of classifiers furnish estimates of parameter influence. Random forests can report a mean decrease in accuracy for each input variable. This measures the loss in accuracy of a tree within the forest when the variable of

interest is removed. Variable importances in deep neural networks are calculated from the network's weight matrix directly (Gedeon 1997). Variables were ranked by relative importance, these ranks averaged across all fitted models (separately for each model) weighted by test-set prediction accuracy (a measure of the goodness of the model). All models were fit using the H20 machine learning platform via the R interface package (Fu et al. 2015). Models were fit with all default parameterizations except 500 trees per forest were used for each random forest.

Role of Phenological Offset:

Phenological offsets were hypothesized to promote coexistence in the presence of high degrees of competitive asymmetry. To test this hypothesis another set of simulations were run where the competitively dominant species was assigned a growth rate of 7%/day, the competitively inferior species was assigned a growth rate of 4% per day (43% slower growth). The species had identical relative attractiveness levels at 41%, the calculated optimum for spore dispersal (Role of Attraction below and appendix 1), and 38 dung pats were deposited per year. The remaining parameters are given in Table 1. The phenological offset for the competitively inferior species ranged from 0 - 75 days, with four simulations at each offset.

Results

Empirical Parameter Estimates:

The parameter estimates show that *S. ampullaceum* has a greater spore yield per cm than *S. pensylvanicum*, despite having considerably fewer sporophytes per cm. *Splachnum pensylvanicum* is considerably more attractive to fly vectors, based upon fly visitation/day than *S. ampullaceum* (Table 2). Dung deposition for moose in the region of the study site was estimated to be 11 droppings per day per moose (Miquelle 1983), the study site straddles two

moose management areas with a mean density of 3.60 moose/km2 (Newfoundland Wildlife Division 2014 unpublished data), however moose tend to avoid roadways by approximately 500 m, with only 20% approaching to within 50 m (Laurian et al. 2008). As our site is between 25 and 300 m from the nearest road, moose density was assumed to be 25% of the management zone average (0.90 moose/km2). Moose were assumed to have season home ranges for the spring and summer of approximately 7.9 km2 (Cederlund and Sand 1994; Kerckhoff et al. 2013). A moose producing 11 droppings at random locations within a home range of 7.9 km2 will produce dung pats within a 0.232 km2 subsection of its home range (equivalent to the area of the simulated peatland) on average 0.29 times per day, or 44 times per 150 day season.

Role of Attraction:

The dispersal output of a moss to any given dung pat over the course of the year depends significantly on the mosses' attractiveness and the amount of time the moss is less attractive than the dung. The number of dispersal days (w) of a moss has to get to any particular dung pat can be given by:

$$w = 1 + \lfloor \sqrt{-2\sigma^2 log A_m} \rfloor \quad (14)$$

And so the dispersal output of a moss to any given dung pat is:

$$\sum_{i=1}^{w} A_m e^{-\frac{i-1}{2\sigma^2}} - A_m^2 \quad (15)$$

This function is optimized at attractiveness values close to 0.41 (Appendix 1 Fig S2)

Simulation Outcomes:

Coexistence occurred in 732 (14.6%) of the simulations from the 5000 latin hypercube samples. For phenology, most coexistence outcomes were observed for the staggered phenologies (Fig. 1),

and the data fall into four major groups; two uniform competitors, two identical sinusoidal phenologies, one sinusoidal and one uniform, and two staggered sinusoidal phenologies. When the two competitors have uniform phenologies, the probability of the two species coexisting is 1.5% (5/334), when the competitors have non-uniform but identical phenologies 1.6% (15/958) of simulations have coexistence. When one competitor has a sinusoidal phenology while the other has uniform phenology, 11.8% (216/1832) of the simulations have coexistence, and when the two competitors have staggered sinusoidal phenologies 26.4% (496/1876) of the simulations have coexistence. Coexistence likelihood appears stable with increasing variance as the difference in attractiveness between the two species increases (Fig 2A), with a mild decrease of 6% over the full range of attractiveness, fit but not depicted. The likelihood of coexistence decreases approximately linearly with increasing differences in competitive ability(growth rates), with no simulations at coexistence differences greater than 5.5%/day coexisting. Coexistence likelihood increases linearly with increasing substrate availability. When parameter space is partitioned by phenological group (Fig. 3-4) the pattern of increasing likelihood of coexistence with increasing phenological mismatch is apparent, asymmetry in phenology is visible as an increase in persistence time throughout the panels, with the shortest persistence times when both competitors have uniform phenology and highest when the two competitors are staggered. Figure 4 shows a pattern of decreasing dependence of persistence time on growth rates difference as phenological asymmetry increases. The effect of diversity was generally to increase the likelihood of coexistence especially when the amount of available substrate was high (Fig. 5).

Machine Learning and Variable Importances

In total 24 predictors were provided to the machine learning models (Table 3) to determine which were most indicative of whether or not both species would coexist. The performance of

the models was consistent across the split sets with mean ± 1 standard deviation prediction accuracy of 90.7 ± 0.7%, with only a minimal difference in accuracy between the two algorithms (Table 4). The AUC for both models was 76.7 ± 3.4% with deep networks performing better with mean 78.5% vs. 75.1% for the random forest. In general, predictors fell into a number of common categories: species competitive ability, species phenology, species attractiveness, substrate availability, and aggregation statistics. Each of these groups consists of various codings of the predictors (e.g. phenologies, phenology pairings, and phenology pair groups). Since the mutual information between codings is high, variable importance depends strongly on the presence of collinear predictors. In the case of random forest, the number of trees was sufficiently high to allow collinear predictors to separate and allow a reasonable estimate of independent importance to be obtained. In the case of the deep neural networks, each predictor occurs in every model and so the importance will be shared between mutually informative parameters with the most informative coding receiving the majority of the importance.

The two algorithms selected very different sets of key predictors, although each algorithm was generally consistent in its rankings across test sets. From the five importance metrics for each variable (overall rank, standardized random forest importance, standardized deep network importance, and mean ranks for both algorithms: Table 5), the difference in growth rate was the most important predictor of coexistence, with a mean rank of 1.30, followed by the two individual growth rates at 3.30 for *S. ampullaceum* and 3.89 for *S. pensylvanicum*. Following this were growth rates with Shannon-Weiner diversity with a mean rank of 5.10, ranking highly in both random forest models and deep networks. Substrate availability had a mean rank of 6.10, with two codings of phenology pairings at 6.60 and 6.85. The highest ranking attractiveness statistic was the attractiveness of *S. ampullaceum* with a mean rank 9.24. The highest ranking

classical aggregation statistic was Sevenster's (1996) *T* statistic measuring the effect of *S. pensylvanicum* on *S. ampullaceum*. Next was Shorrocks and Sevenster's (1995) *A* statistic, which ranked highest in the deep network models of all statistics while ranking lowest in random forest models. The higher ranks were populated by an assortment of aggregation statistics, their between year differences, and attractiveness measures and differences. Ranking lowest were the individual phenologies of the two species.

Effect of Phenological Offset:

In the experiment examining the focused effect of staggering on two highly asymmetric competitors, increasing the number of days between peak spore production (ν_m) increased the mean number of years coexisted approximately exponentially, reaching the threshold of indefinite coexistence in all simulations with greater than a 53 day stagger when the competitive difference was 50%, and 37 days when the competitive difference was 25% (Fig. 6). Increasing phenological staggering had a monotonically decreasing effect on the observed Shannon-Weiner diversity of the populations (Fig. 7).

Discussion

Our modeling results suggest that next to competition itself, the availability of substrate and the degree to which competitors become aggregated intraspecifically are the most influential in differentiating which simulations had coexistence from those that did not. In general, it appears that aggregation is a phenomenon that captures a large amount of the variability in dispersal, particularly from phenological differences, acting to modulate the likelihood of coexistence. This is supported by the high importance ranking for Shannon-Weiner diversity in both types of models, and the primacy of Shorrocks and Sevenster's (1995) *A* statistic in the deep neural network models. Excluding the other aggregation measures, both diversity and the *A* statistic

correlated most strongly with phenology differences and substrate availability, suggesting that these statistics are capturing an emergent property of dispersal difference between the two moss species. Attractiveness appeared to play a very weak role determining coexistence outcomes, with a highest mean rank of 9.24 for *S. ampullaceum*'s attractiveness, and a rank of 15.04 for the difference in attractiveness.

This work revisits and extends that of Marino (1991), providing phenology as a causal mechanism for the spore aggregation he had shown to be capable of permitting coexistence in simple simulations. We put phenology forward as the causal agent because of the clean monotonically decreasing relationship that increasing phenological stagger has with observed Shannon-Weiner diversity (Fig. 7). Although growth rates, diversity, and substrate availability were stronger predictors overall, we believe that phenology is critical in allowing coexistence between these two mosses in peatlands in Newfoundland. At competitive differences of 25% and 50% the phenological offsets required to go from no simulations having coexistence, to all simulations having coexistence was 37 and 53 days respectively when there were 38 dung pats available per year. These required offsets are shorter than the stagger we observe between *Splachnum ampullaceum* and *S. pensylvanicum* in Newfoundland. At lower substrate availability and greater competitive differences greater staggers are likely needed This is especially evident given that the highest proportion of coexistence, at the maximum offset of 75 days was only 30%, so additional mechanisms need to be at play to ensure coexistence. The effect of phenology separates clearly into three groups, simulations with identical competitors, simulations with one sinusoidal and one uniform competitor, and simulations with staggered sinusoidal competitors. Simulations where competitors were identical had less than 2% chance of reaching indefinite coexistence. This indicates that only under the most permissive combinations of the other

parameters is coexistence possible without some form of phenological asymmetry. Simulations where competitors were not identical had greatly increased chances of coexistences. When one competitor had a uniform instead of a sinusoidal phenology, the proportion of simulations coexisting increased to just fewer than 12%, whereas the proportion of simulations with staggered phenologies increased to 26.4%. The staggers in the phenology pairs were 37.5 days and 75 days, so the effect of a uniform competitor is equivalent to a phenological offset of less than 37.5 days. It seems probable that the effect of staggering can be captured by the integral of the absolute difference between the two phenology curves, which would place the effect of a uniform competitor equivalent to a phenological offset of about 25 days. The finding that phenological staggering can promote coexistence corroborates the results of Nakazawa and Doi (2012) and Revilla *et al.* (2014), suggesting, in addition, that competitors need not necessarily be staggered with respect to their resource, but potentially staggered with respect to their competitor's resource usage phenology instead.

The amount of available substrate was also highly influential in influencing coexistence outcomes with a mean rank of 6.4. No simulations had coexistence when there were fewer than 8 dung pats deposited per year. This certainly matches the authors' (unpublished) observations of sparsely occupied peatlands typically dominated, or even monopolized, by *S. ampullaceum*. Altering the number of available dung pats per year has several key effects on the metacommunity. Firstly, it determines the amount of available space to be competed for, this has been hypothesized to promote species richness in other metacommunities (Fahrig 2003; Fahrig 2013), secondly, it decreases the inter-patch distances, facilitating dispersal which has been shown in other epiphytic moss species to increase metacommunity size (Snäll et al., 2005), and lastly it facilitates the effects of phenological staggering by increasing odds that fresh dung will

be available during both species' dispersal optima.

No single variable dominated in terms of influence on coexistence outcomes; in both algorithm types the relative importance of the top ranked predictor was approximately 10% of the sum of all importances. There are parameter levels that preclude coexistence, in addition to the 8 dung pat boundary, no simulations with a growth rate difference of greater than 6%/day achieved indefinite coexistence. There were no parameter levels in the univariate case that guaranteed coexistence. The coexistence outcome dependence on several parameters is evident in the irregularity in 2-dimensional snapshots of the coexistence-time response surface (Figs 3 and 4) indicating, that for simple two species meta-communities, outcomes are not predictable without considering a suite of predictors. This highlights the importance of considering the inherent complexity of ecological systems, and the value of using machine learning approaches to address situations where high dimensionality and or nonlinearity limits the inferential and predictive power of traditional approaches.

The two machine learning techniques disagreed as to which predictors were most important; the most striking example being the ranking of the *A* statistic. The types of relationships a neural network is capable of learning differs from those learned by random forests. For a comparison of the patterns learned by the two machine learning techniques see appendix 2, in essence the importances deduced by the random forest behave somewhat like variance explained, where the importances learned by the neural network is less intuitive (Gedeon 1997; Breiman 2001; Louppe et al. 2013).

When considering the effect of aggregation in a two species metacommunity the best metric for predicting coexistence was the Shannon-Weiner diversity. Although it is worth noting that Shorrocks and Sevenster's (1995) *A* statistic was the top predictor in the deep learning models.

Since the *A* statistic is calculable from combinations of the other predictors, it is perhaps unsurprising that trees in the random forest were able to perform well without it. The *A* statistic likely performed well in the deep network models because it captures most of the important information regarding realized dispersal. The statistic includes both intraspecific (*J* statistics for both species) and interspecific (*C* statistic) forms of aggregation. The degree of aggregation (of both types) is controlled exclusively by dispersal, as the statistics were calculated for only years one and two (before the action of competition). The degree of aggregation, in turn, is controlled by: the amount of available substrate, the phenology of both species, and their relative attractiveness. It even captures some of the stochastic variability in substrate location (although only for the first two years). Shannon-Weiner diversity instead measures the degree to which the two species segregate amongst the patches, capturing effective competition. The only other aggregation statistics to rank highly in the over-all rankings were Sevenster's (1996) *T* Statistics. These rated in the top half of predictors for both the neural networks and the random forests, although in both cases they were outranked by other aggregation statistics. It is important to note that contrary to Sevenster's observation, the *T* statistics were not sufficient criteria for identifying simulations where coexistence would occur.

The attractiveness of either moss species, contrary to the authors' intuition as gained through detailed knowledge of their natural history, failed to rank as highly as growth rate, phenology, or substrate availability. The attractiveness of *S. ampullaceum* and *S. pensylvanicum* had mean ranks 9.24 and 13.04 respectively. There was a weak negative trend in the attractiveness difference results, with coexistence probabilities decreasing 6% over the full range of attractiveness differences compared to changes of over 30% for growth rate differences and substrate availability. Prior to this study it was hypothesized that the mosses were under

balancing selection for an optimum attractiveness that maximized the length of time they were attractive: 0.41% as attractive as fresh dung, however it was determined that the mosses (albeit from a single sampling month) were considerably more attractive than the expected optimum. This observation and the simulation results suggest that attractiveness differences may play only a minor role in determining coexistence outcomes. In these simulations, a higher attractiveness leads to a shorter dispersal window that is more productive per unit time; under a model of stronger preemptive competition it would perhaps be advantageous to maximize dispersal earliest in the colonization window of the dung pat.

This study shows that coexistence in a two species meta-community of Splachnaceae mosses is dependent on multiple factors; ideally competitors should be similar in terms of growth rate, with a maximal amount of offset between the dispersal maxima. The degree to which the two species exclude one another is captured by their competitive asymmetry and the degree to which they become intraspecifically aggregated. This is likely true for other patch based metacommunities. Aggregation and competitive ability act in concert and are likely the two most influential determinants of coexistence outcomes. It seems likely that the Splachnaceae moss coexisting in Newfoundland peatlands are doing so by optimizing their phenological staggering, with *S. ampullaceum* producing one large, mid-season sporophyte flush, and *S. pensylvanicum* producing an early and late flush.

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Figures

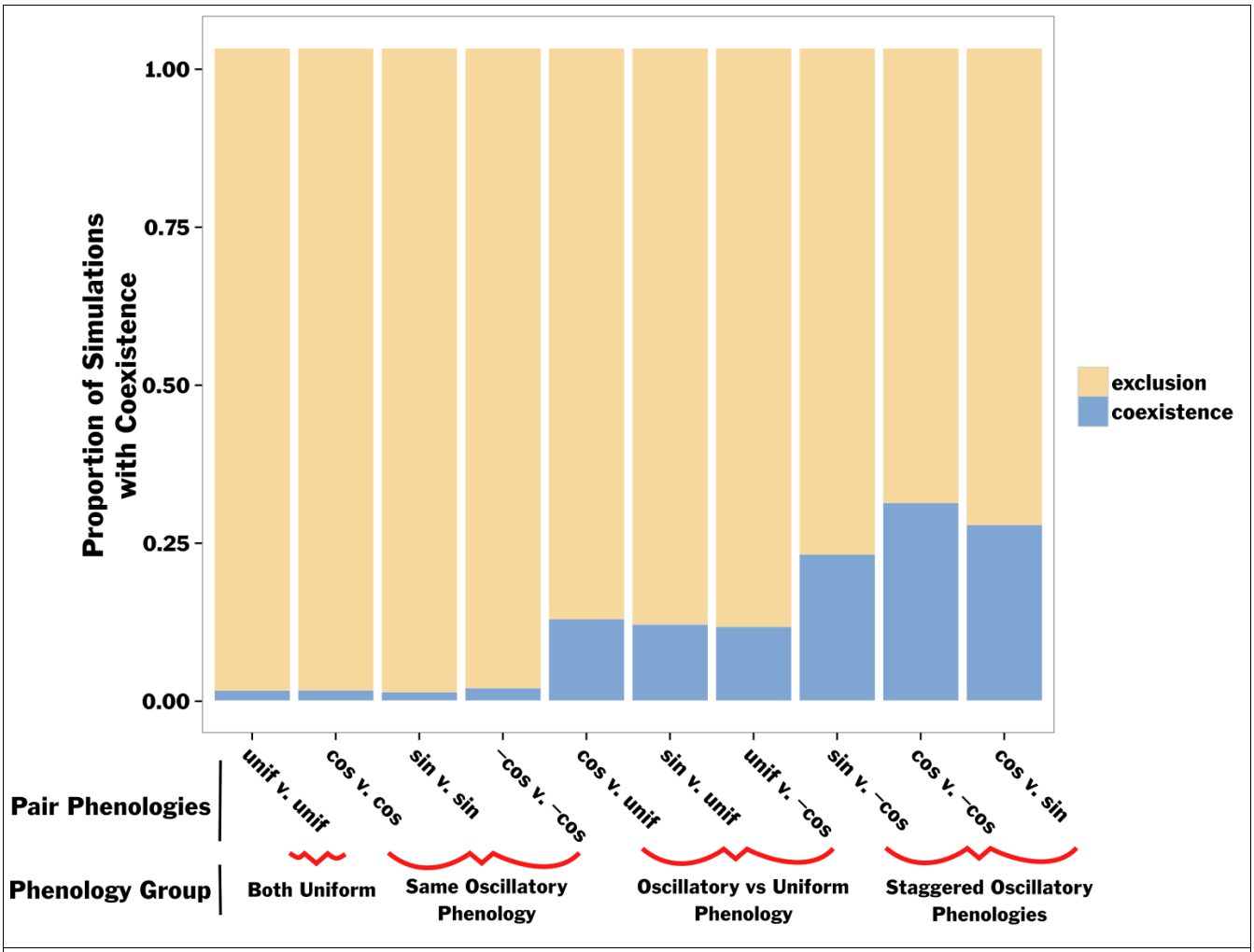

Fig 1. Proportion of simulations with coexistence given competing phenologies.

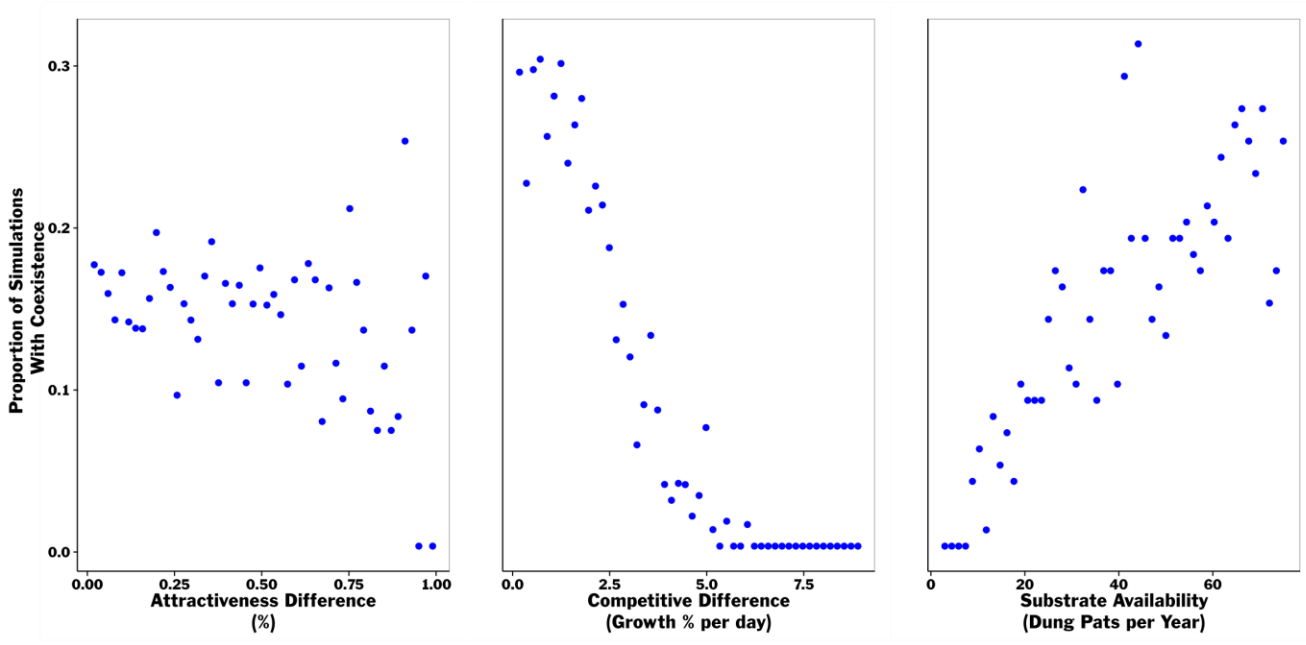

Fig 2. Proportion of simulations with coexistence given attractiveness difference (panel A), competitive difference (panel B), and substrate availability (panel C). The observations were binned into 50 evenly spaced groups over the range of the x-axis to determine proportion of coexistence.

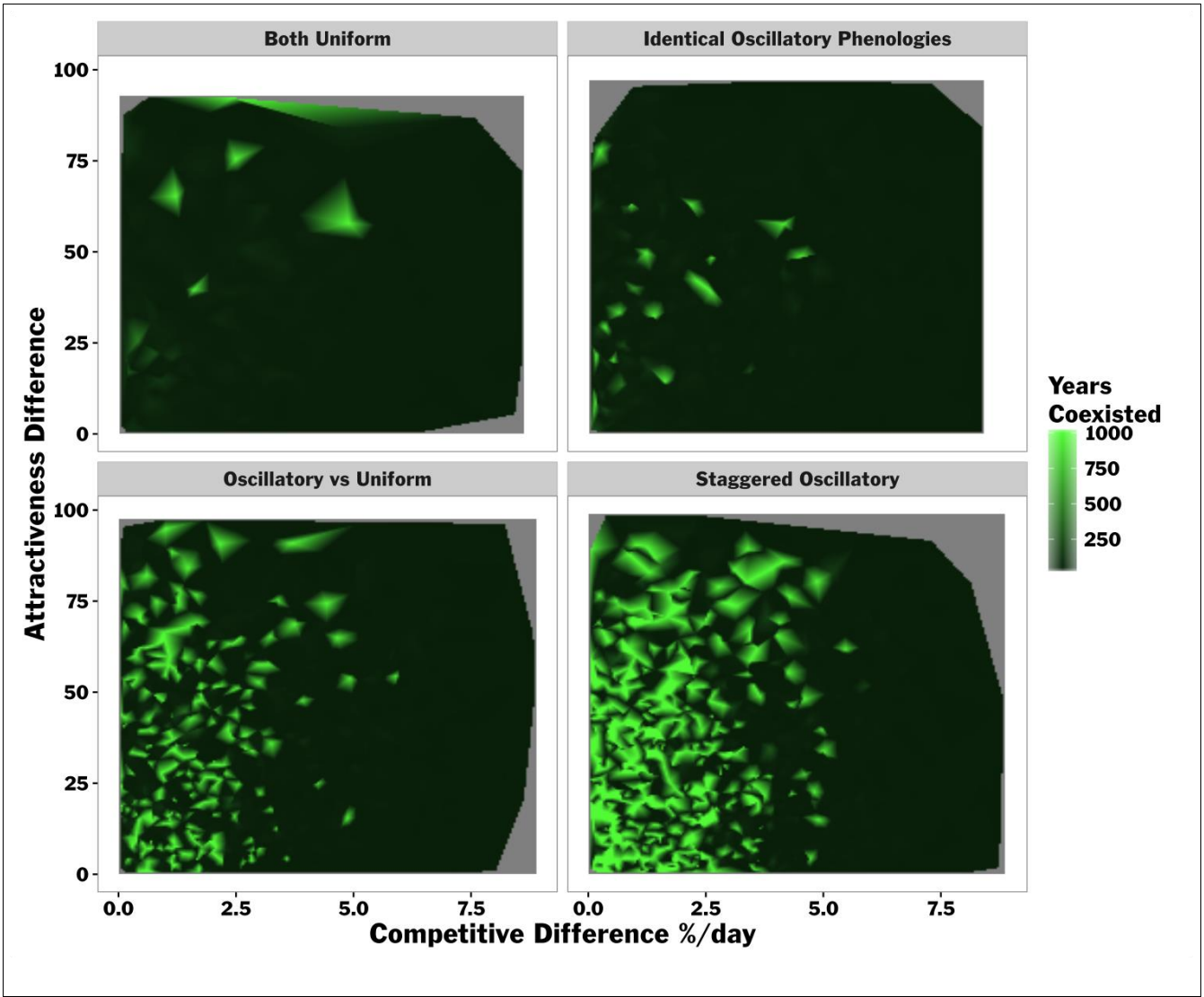

Fig 3. Years coexisted contour plot exploring parameter space in terms of competitive difference and attractiveness difference

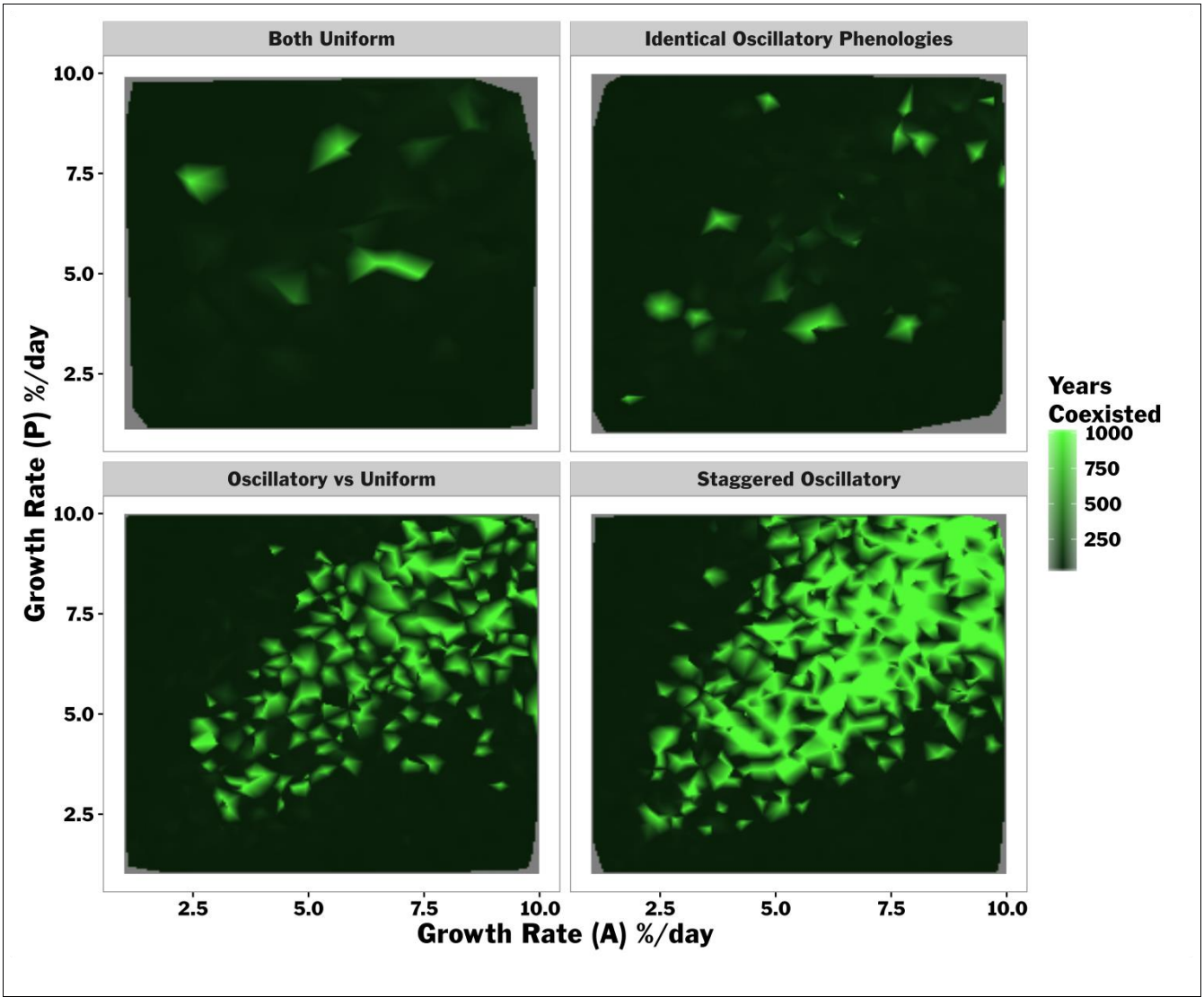

Fig 4. Years coexisted contour plot exploring parameter space in terms of the two species growth rates

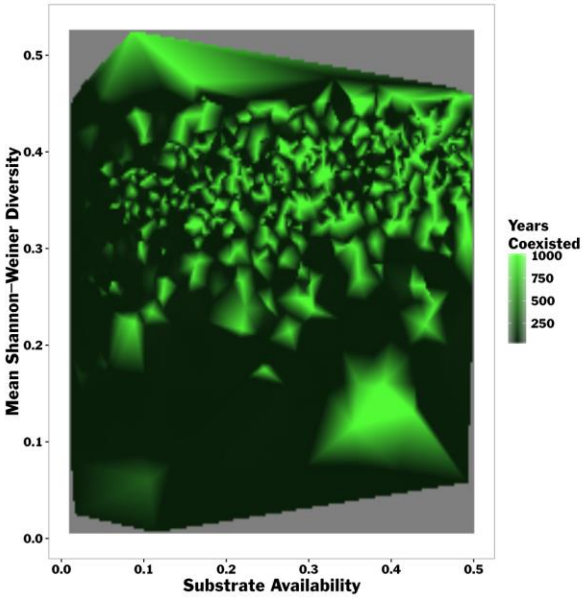

Fig 5. Contour plot showing the mean number of years coexisted for two phenologically staggered competitors given the substrate availability and the observed average Shannon-Weiner diversity of populations in years one and two.

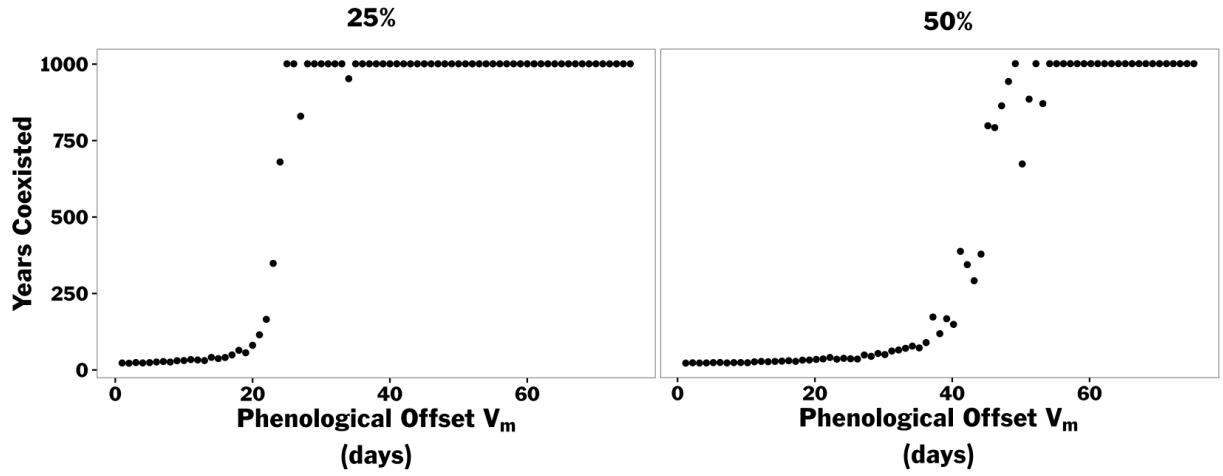

Fig 6. Influence of Phenological staggering on coexistence time in competitively asymmetric competitors. Points represent mean number of years coexisted for three simulations at each phenological offset. The left panel shows the results for simulations with the superior competitor growing 25% faster, the right panel shows results for simulations where the superior competitor growing 50% faster.

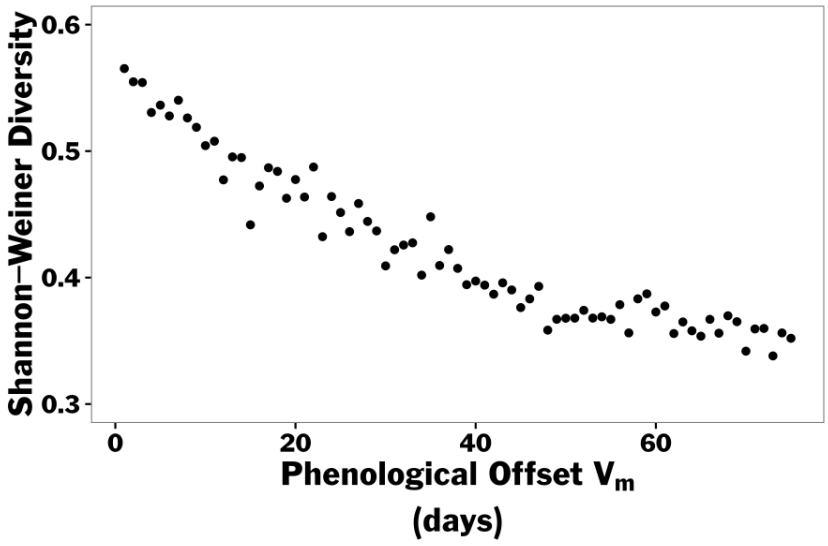

Fig 7. Effect of increasing phenological staggering of observed Shannon-Weiner diversity in years one and two. Points represent the mean diversity for three simulations at each phenological offset.

Table 1: Default (non-varied) parameter values for all simulations

Parameter	Value
Peatland radius	282m
Days per growing season	150 days
Simulation length	1000 years
Competition Coefficients (α_{12} and α_{21})	1
Areal Yield* (Y)	0.10
Attractiveness decay mean (μ)	0 days
Attractiveness decay standard deviation (σ)	2 days

* Areal yield is the amount of substrate one areal unit of mature moss can colonize on dung immediately adjacent in one day during its phenological peak.

Table 2: Table of empirical parameter estimates

	Species	
Parameter	*S. ampullaceum*	*S. pensylvanicum*
Sporophytes per cm	6.13 ± 3.12	16.94 ± 4.55
Spores per sporophyte	30,000 ± 9000	3500 ± 1800
Approximate yeild per cm	183,900	59,290
Relative Attractiveness	0.52	.76

Table 3: Meaning of predictors supplied to machine learning algorithms

Predictor	**Meaning**
Growth Rate (A)	Growth rate in %/day for *S. ampullaceum* (0.01 - 0.10)
A Statistic	Shorrocks and Sevenster's A statistic representing the effect of intraspecific aggregation relative to interspecific aggregation
Phenology Group	The four class grouping system used to aggregate phenology pairs. Includes: both uniform, two identical oscillatory, one oscillatory one uniform, and staggered oscillatory phenology.
Mean Diversity	The average diversity across all dung pats colonized in year one or two (prior to competitive interactions.
Growth Rate (*P*)	Growth rate in %/day for *S. pensylvanicum* (0.01 - 0.10)
Substrate Availability	Number of new dung pats made available per growing season (2 -75)
Phenology Pair	The exact phenological pairing (irrespective of which species has which phenology)
Attractiveness (A)	The attractiveness of *S. ampullaceum* (0.01 - .99)
T_{AP}	Sevenster's *T* (influence of *S. pensylvanicum* on *S. ampullaceum*)
T_{PA}	Sevenster's *T* (influence of *S. ampullaceum* on *S. pensylvanicum*)
Attractiveness (P)	The attractiveness of *S. pensylvanicum* (0.01 - .99)
Mean *J* Statistic	Overall Mean J statistic for both species
Phenology (P)	The phenology of *S. pensylvanicum* (uniform, sin, cos, -cos)
J Statistic (A)	Ives *J* Statistic representing intraspecific aggregation (for *S. ampullaceum*)
J Statistic (P)	Ives *J* Statistic representing intraspecific aggregation (for *S. pensylvanicum*)
Competitive Difference	Difference between the growth rates of *S. ampullaceum* and *S.*

	pensylvanicum
Phenology A	The phenology of *S. ampullaceum* (uniform, sin, cos, -cos)
C Statistic	Ives' *C* statistic representing overall interspecific aggregation
Difference in *C*	Difference between year 1 and year 2 estimates for *C*
Difference in Attractiveness	Difference between the attractiveness of *S. ampullaceum* and *S. pensylvanicum*
Difference in *J* (P)	Difference in year 1 and year 2 estimates for the *J* statistic of *S. pensylvanicum*
Difference in *J* (A)	Difference in year 1 and year 2 estimates for the *J* statistic of *S. ampullaceum*
Difference in Diversity	Difference in year 1 and year 2 estimates for patch diversity
Simulation Code	Dummy id variable for each simulation

Table 4: Model performance metrics for coexistence prediction model. Prediction accuracy and area under the receiver-operator characteristic curve (AUC) for both of machine learning algorithms for each of the ten training and test set pairs.

		Result Set Division										
Metric	Algorithm	1	2	3	4	5	6	7	8	9	10	Algorithm Mean
Accuracy	Deep Neural Network	0.903	0.913	0.901	0.906	0.898	0.921	0.902	0.902	0.910	0.895	0.905
	Random Forest	0.905	0.911	0.911	0.915	0.916	0.917	0.900	0.896	0.906	0.911	0.909
	Set Mean	0.904	0.912	0.906	0.911	0.907	0.919	0.901	0.899	0.908	0.903	0.907

AUC	Deep Neural Network	0.755	0.808	0.770	0.762	0.772	0.835	0.761	0.766	0.816	0.802	0.785
	Random Forest	0.742	0.809	0.761	0.795	0.751	0.751	0.727	0.706	0.716	0.751	0.751
	Set Mean	0.748	0.809	0.766	0.778	0.762	0.793	0.744	0.736	0.766	0.777	0.768

Table 5: Machine learning importance metrics for prediction of coexistence within a simulation. The average ranks are the accuracy weighted average of predictor ranks for each of the ten models of both types. Importances are the mean importance across the ten models of each type weighted by the accuracy of the model. Individual model importances are scaled such that the importance of all predictors sums to one.

Predictor	Average Rank	Average Random Forest Rank	Average Random Forest Importance	Average Deep Network Rank	Average Deep Network Importance
Competitive Difference	1.30	1.00	0.1000	1.60	0.0949
Growth Rate (A)	3.30	2.70	0.0663	3.90	0.0723
Growth Rate (P)	3.89	2.30	0.0671	5.50	0.0699
Mean Diversity	5.10	5.80	0.0373	4.40	0.0712
Substrate Availability	6.40	4.00	0.0544	8.81	0.0639
Phenological Grouping	6.60	7.30	0.0303	5.90	0.0681
Phenology Pair	6.85	5.20	0.0386	8.50	0.0639
Attractiveness (A)	9.24	8.10	0.0275	10.38	0.0618
T_{AP}	11.90	10.20	0.0226	13.61	0.0604
A Statistic	12.72	24.00	0.0056	1.40	0.0978

Attractiveness (P)	13.04	8.60	0.0264	17.49	0.0576
T_{PA}	13.90	13.20	0.0192	14.60	0.0590
C Statistic	13.90	13.20	0.0192	14.59	0.0590
J Statistic (P)	14.46	16.10	0.0172	12.81	0.0601
Overall Mean *J* Statistic	14.96	16.50	0.0171	13.41	0.0597
Attractiveness Difference	15.04	10.80	0.0219	19.32	0.0560
Diversity Difference	15.56	18.20	0.0163	12.90	0.0607
J Statistic (A)	15.65	16.90	0.0169	14.41	0.0591
Difference in C	16.80	19.70	0.0159	13.88	0.0595
Simulation Code	18.29	12.60	0.0196	24.00	0.0490
Difference in *J* (P)	19.45	20.40	0.0156	18.51	0.0570
Difference in *J* (A)	19.45	18.20	0.0163	20.70	0.0558
Phenology (A)	20.55	22.10	0.0100	18.99	0.0562
Phenology (P)	21.65	22.90	0.0090	20.40	0.0555

Chapter 3: Examining Dispersal and Competitive Abilities of *Splachnum ampullaceum* and *S. pensylvanicum*

Abstract

Dispersal and competitive ability were compared in two entomophilous Splachnaceae mosses, *Splachnum ampullaceum* and *S. pensylvanicum* to assess the likelihood that a competition-colonization trade-off between them promoted their coexistence. The two mosses occur in pure and mixed populations. Three dispersal experiments were conducted to assess if there was any evidence of dispersal limitation, if species differ in dispersal ability, and if spore transfer depends on distance at the intra-peatland scale. A response surface designed competition experiment was used to determine the competitive differential between the two species and if it depends on a gradient from the middle to the outside of the patch which corresponds to a moisture gradient. *Splachnum ampullaceum* is generally the superior competitor with 58.6% more gametophytes across all sowing treatments. However, the competitive dominance of *S. ampullaceum* shifted to *S. pensylvanicum* towards the drier edges of dung pats. Both species shared a benefit at high sowing density potentially indicating interspecific facilitation at high gametophyte density. We found no evidence of dispersal limitation, that the two species growing alone differ in spore dispersal ability or differ from mixed populations at the intra-peatland scale. Evidence was found that dispersal decreases as a function of distance from the nearest population. Both species shared a benefit at high sowing density potentially indicating interspecific facilitation at high gametophyte density.

Introduction

Coexistence between competing species is an enduring question in ecology. The traditional view

is that species sharing many ecological properties are unable to coexist, as the competitor whose population can grow on the lowest level of their mutually limiting resource will deterministically exclude its competitor (Tilman 1982; Hardin 1960). Modern research has expanded on the suite of potential limiting resources and identified coexistence mechanisms that can permit coexistence of all but the most identical species (Amarasekare 2003; Chesson 2000). Coexistence mechanisms can be broadly classified into either stabilizing or equalizing mechanisms. Stabilizing mechanisms act to reduce the degree to which each species pair competes for their most limiting resource, whereas equalizing mechanisms act to decrease the competitive discrepancy between competing species (Chesson 2000). Coexistence is, however, context dependent (Schmitz 2010), consequently, the key mechanisms that promote coexistence in a given system remains an open problem for many communities and ecosystems.

A classic null hypothesis when confronted with presumably improbable coexistence is the competition-colonization trade-off (Calcagno et al. 2006; Tilman 1994) where the inferior competitor avoids competition by dispersing further and/or faster than the superior competitor. A useful model system for looking at the competition-colonization tradeoff is the entomophilous mosses in the family Splachnaceae. These mosses are restricted to growing on organically enriched substrate and form distinct reproductively isolated populations on patches of dung or carrion (Marino et al. 2009). Marino (1991a; 1991b) observed that often species of entomophilous Splachnaceae can be found growing in single and mixed-species populations on individual droppings within seemingly identical habitats. On the island of Newfoundland, Canada, peatlands contain two species of Splachnaceae, *Splachnum ampullaceum* Hedw. and *S. pensylvanicum* (Brid.) Grout ex H.A. Crum. These two species occur in pure and mixed populations, competing for space on the summer droppings of moose (*Alces alces* L). Hammill

and Marino (2016) used a simulation model to assess how these two highly similar species can circumvent the competitive exclusion principle; however, their model did not include empirically estimated competitive and dispersal parameters. Here we attempt to fill this knowledge gap by determining how the mosses compare in terms of dispersal ability by trapping spores moving throughout peatlands, and competitive ability using a response surface designed in lab growth experiment.

Bryophytes in general are ideal for studying patch-related coexistence. They tend to have broad distributions enabling phylogeographic comparisons of effects on an inter-continental scale, many exhibit strong habitat specificity, they tend to have short generation times, and patches turnover occurs on a reasonable temporal scale allowing experimentation (Pharo and Zartman 2007). Within the bryophytes, the predominant modes of dispersal are reliant on wind or water to transport spores to new substrate. These forms of spore transport are inefficient, with many spores being carried to unsuitable substrate. This inefficiency is likely the source of the evolutionary pressure for Splachnaceae to have evolved their complex dispersal syndrome and deceptive signaling (Marino and Raguso 2016). The deception evolved by Splachnaceae is brood site mimicry (Urru et al. 2011), with the mosses emulating their substrates primarily with olfactory signals with varying degrees of fidelity, resulting in differing fly vector faunas (Marino and Raguso 2016, Marino et al. 2009). It is unknown currently to what degree different vector faunas contributed to differing dispersal potential.

In this study, we assessed whether a competition-colonization trade off exists for *S. ampullaceum* and *S. pensylvanicum*. We explored relative competitive abilities across a variety of competitor ratios and densities using a response surface design (Inouye 2001) and we determined if the two mosses differed in their dispersal ability at the intra-peatland scale. We additionally examined

whether substrate colonization was dispersal or substrate limitation. We hypothesized, following Cameron and Wyatt (1986) that Splachnaceae would be substrate limited, with effective spore dispersal at the intra-peatland scale. We also hypothesized, based its greater abundance in the field, that *S. ampullaceum* was the superior competitor, and that there was a competition-colonization trade-off such that *S. pensylvanicum* was a more effective disperser, either in crude magnitude or improved dispersal at longer distances.

Methods

Splachnaceae Ecology:

The family Splachnaceae is unique among the bryophytes in having animal mediated dispersal that uses active attraction of vectors (Marino *et al*. 2009). Approximately 50% of Splachnaceeae species are entomophilous, and use flies (Diptera) to disperse their spores. Species using this dispersal strategy are typically restricted to growing on dung and carrion (Marino et al. 2009). To attract spore-dispersing flies, the mosses, which provide no nutritional reward to flies, use both olfactory and visual signals to manipulate fly behaviour, deceptively attracting flies seeking dung or carrion either for food or oviposition sites (Marino and Raguso 2016). Flies typically spend between 2 and 7 minutes interacting directly with the sporophytes (Cameron and Wyatt 1986) during which time sticky clumps of spores adhere to the flies, which may then be dispersed to fresh dung. The intense substrate specificity of the entomophilous Splachnaceae is hypothesized to be primarily due to this relationship. In contrast to most mosses, wind is not effective at dispersing spores of entomophilous Splachnaceae, likely because the spores form sticky clumps (Cameron and Wyatt 1986).

The geographic distribution of the two species differs; *Splachnum pensylvanicum* is primarily a

North American species found growing from Florida to Newfoundland east of the Appalachian Mountains, although specimens reported as *S. pensylvanicum* have been observed in Brazil (Lüth and Goffinet 2005). Only in the extreme northern limit of its ranges does the distribution of *S. pensylvanicum* overlap with the range of moose. In contrast, *S. ampullaceum* is a circumboreal species whose range overlaps widely with that of moose. The visual and the olfactory signals of the two species differ. Sporophytes of *S. pensylvanicum* have small setae (4 - 9 mm) and a green, red distally, hypophysis. *Splachnum ampullaceum* has larger yellow/pink sporophytes with setae between 15 and 65 mm. The two species are both reliant on fly dispersal vectors and share overlapping but distinct vector taxa, and the two species differ in odour chemistry (McCuaig et al. 2014; Marino and Raguso 2016).

Competition Experiment:

We examined the influence of varying the spore concentration and the proportion of sown spores of each species (competitor ratio) on competitive outcomes, moss spores were sown according to a response surface design (Inouye 2001). The response surface design differs from a simple De Witt replacement series (Inouye and Schaffer 1981) by varying the overall sowing density along with the ratio of the two competitors. Three total moss spore concentrations were used one million, one hundred thousand, and ten thousand spores per mL), the competitor proportions were either both zero or complimentary proportions of 0, 20, 40, 60, 80 and 100% of each competitor, which is a complete DeWit replacement series at each sowing density. Two replicates of the whole DeWit series at each sowing density were performed. Spore inoculla were sown on 100g of moose dung (frozen from fresh until use) in individual perforated weigh boats. The weigh boats were placed in trays, each tray containing all input proportion treatments for a given replicate and spore concentration. The trays were kept filled with water to allow the dung

to absorb water through the perforations in the weigh boats thus keeping the mosses moist throughout the experiment. We also watered the upper surface of the dung via a spray bottle whenever the dung/mosses began to dry.

In order to compare the competitive ability of the two mosses in the growth experiment, we identified gametophytes along three edge-to-edge transects with a 1 cm spacing grid for a total of 12 sites per transect. The position of each gametophyte (or absence thereof) was grouped into one of three categories; edge (outer two sites on each side), mid (next two inward on each side), and center (the inner-most four). Position on the plate was used as a proxy for moisture gradient as plates were observed to dry from the edges inward.

Analysis:

To estimate competitive abilities, we first compared the proportion of counted gametophytes that were *S. ampullaceum* vs. *S. pensylvanicum*. To assess the dependence of competitive ability on sowing density, input proportion, and grouping factors, each counted site was coded as *S. ampullaceum*, *S. pensylvanicum*, or uncolonized and fit with a multinomial regression. All ratio scale terms were scaled to range between 0 and 1 such that effects could be compared between ratio scale predictors and categorical predictors. Since the combinatorics of producing all possible models from a set of predictors is unfeasible, we performed a first pass model selection to determine which features should be included in the analysis and whether to include interactions. All combinations of the four main predictors: concentration, proportion of input spores of *S. ampullaceum*, region of the plate, and tray were assessed either including all interaction terms or including none. The proportion of input spores of *S. pensylvanicum* was too highly collinear with the input spores of *S. ampullaceum* to fit the multinomial regression, as together both summed to one except for the controls, and was excluded from model fitting to

avoid matrix singularity. The fully interactive model including concentration, input spores of *S. ampullaceum* , and region had an Akaike weight of greater than 99% so only nested sub-models of this were considered for the final stages of the analysis. We used model selection to identify from these nested sub-models the most parsimonious set of explanatory variables for gametophyte identity. We used the second-order bias-corrected version of Akaike's an information criterion (AICc) to rank alternative models (Hurvich and Tsai 1989; Burnham, Anderson, and Huyvaert 2011) in both model selection steps. No *a priori* thresholds were considered, instead we examined the evidence ratios to exclude unsuitable models.. The fixed effects in the best model (as chosen by AICc) were tested with type 3 analysis of deviance using the package *car* (Fox and Weisberg 2011); this form of analysis of deviance allows effects to be tested irrespective of higher order interactions. Nagelkerke's (1991) pseudo-R^2 was used to assess the quality of fit for each model. Nagelkerke's psuedo-R^2 is the familiar Cox and Snell (1989) pseudo-R^2 normalized to range from 0-1. All models were fit using multinom from the *nnet* package (Venables and Ripley 2002).

Spore Movement:

Location and Substrate:

We conducted all dispersal experiments in peatlands near Salmonier Nature Park, Holyrood, NL (47º 15.037' N, 53º 18.424' W). Moose dung was obtained from a captive moose at Salmonier Nature Park, dung was collected fresh and frozen until use (with freshly thawed dung used for each trapping session).

Assessing dispersal or substrate limitation:

To determine if Splachnaceae are dispersal or substrate limited, we placed ten 15 mm diameter weigh boats containing ~100g of moose dung in a peatland approximately 10 m from

populations of either *S. ampullaceum*, *S. pensylvanicum* or mixed-species populations. The amount of dung used is considerably smaller than an average moose dung pat. Each dung dish was placed adjacent to a different population, the species composition of each population was not recorded. We collected the plates the following day (< 24 hours later) and placed them in a growth chamber to allow dispersed spores to germinate and grow. The plates were grown on a 12/12 hr light/dark cycle at 2000 μmol/m^2/s, with a 22/15 °C temperature cycle for 3 months. Dispersal limitation would manifest as spores failing to reach dung and hence no gametophyte growth, high colonization success would be taken as evidence for spore dispersal.

Spore trapping experiments:

To examine the importance of entomophilous spore dispersal and the distance with which spores can be dispersed within a peatland, we performed two spore trapping experiments, one to examine the species differences in spore transfer, and one to examine the distance spores travel irrespective of species. We chose peatlands for their absence, within 50m of the experiment region, of wild populations of *S. ampullaceum* and *S. pensylvanicum* to minimize the likelihood of contamination. The presence or absence of wild populations was assessed by a thorough search of the peatland and surrounding areas. Traps were placed either on a small pat of moose dung (baited) to attract flies or left on the surface of the surrounding substrate (for the most part *Sphagnum)* to receive only airborne spores. All unbaited traps were placed 1m from the experimentally transplanted populations to increase the chance of detecting anemophilous transfer if it occurred. We allowed traps to accumulate spores for approximately 24 hours before collection and subsequent analysis. Trapping was repeated weekly over five weeks, stretching from mid-July to late August 2013. Spore traps consisted of molten petroleum jelly coated microscope slides, the cooled petroleum jelly covered a 55mm x 25mm zone on each slide.

For the first experiment, henceforth species experiment, we established three test regions via moss transplantation within one sample peatland. Each region corresponded to one of the three treatments: pure *S. pensylvanicum*, pure *S. ampullaceum*, or a mixed-species populations. Each test region consisted of four transplanted populations forming a 60m x 60m diamond. Spore traps were placed either 1m away from a population with mature sporophytes at the center of the diamond (30m from all populations). See appendix three, panel A for a schematic representation.

For the second experiment, henceforth distance experiment, a single mixed population was placed in a different peatland, also thoroughly searched to ensure that there were no nearby populations of Splachnaceae. We placed traps, two baited and one not baited at 1m, and two baited traps at both 30m and 75m away from a single population to observe how spore transfer changes with distance from the nearest population. The source population was occasionally replaced with a transplant to ensure spore production throughout the trapping period. See appendix three panel B for a schematic representation.

In both experiments, we isolated spores from traps by scraping the petroleum jelly into 1.5 mL microfuge tubes along with 200 µL water. 500 µL diethyl ether was added to dissolve the petroleum jelly and tubes were shaken for 2 minutes at 1600 rpm. Tubes were then centrifuged at (12,000 rpm) to pellet spores. The supernatant ether phase was discarded and tubes were left open 30 minutes to allow residual ether to evaporate. Spores were re-suspended in the remaining water by vortexing and then were counted using a hemocytometer.

Analysis:

For both experiments we fit linear mixed effects models with Poisson errors and a log link and with spore count as our dependent variable as described below. In the species experiment, fixed

effects were distance to nearest population, whether that population was a single or a mixed-species population, and whether or not the trap was baited with dung. For the distance experiment fixed effects were distance to the population and whether or not the trap was baited with dung. For both experiments sampling week and individual trap were random intercept random effects. Model selection was performed as in the competition experiment. We report coefficients of determination for the models following Nakagawa and Schielzeth (2013). We fit mixed effects models with lme4 (Bates et al. 2014a; Bates et al. 2014b) using R (R Core Team 2014)

Results

Spore Movement

Assessing dispersal or substrate limitation:

All 10 dung plates placed into the field for 1 day to determine whether Splachnaceae were dispersal or dung limited were colonized by Splachnaceae spores and rapidly produced gametophytes in the growth chamber (all plates showed signs of growth in under one month).

Spore trapping experiments:

For the species experiment, we attempted to disentangle the role species and distance have on spore dispersal. The most parsimonious model for predicting the number of spores on a given trap included only whether or not the trap was baited with dung as a fixed effect (model 1.1; Table 1a), this fixed effect explains 9.1% of the total variability of the data (marginal R^2) and together with the random effects they explain 50% of the total variability (conditional R^2; Table 1a; model 1.1). The second most parsimonious model incorporated the distance to the nearest moss community slightly increases the marginal R^2, however, it failed to decrease the AICc and

is only 66% as supported by the data as shown by the Akaike weight (Table 1a; model 1.2). The third most parsimonious model (Table 1a; model 1.3) includes only the random effects and has a conditional R2 of 47%, this model is 41% as supported by the data compared to the most parsimonious model. No model with considerable evidence contained the species identity of the nearest population as a predictor of the number of trapped spores. Examining the fixed effects in model 1.1 (Fig 1; panel A) shows the effect of baiting a trap increased the expected number of spores 8.3 – fold. For model 1.2 the effect of baiting a trap increased the expected number of spores 10.2 – fold. The effect of being 30m away from the nearest population decreased the expected number of spores by 35%.

For the distance experiment, in which we isolated the effect of distance, the most parsimonious model was the full model including both distance and whether the trap is baited (Table 1b; model 2.1), the fixed effects accounts for 25.6% of the variability in the data, and, in conjunction with random effects, they account for 69.7% of the variability. The second most parsimonious model (Table 1b; model 2.2) included only whether or not the trap was baited and was 51.3% as supported as the full model. Neither of the other two models had substantial support (Table 1b; models 2.3 and 2.4). Examining the fixed effects in model 2.1 (Fig. 1; panel B) show the effect of baiting the trap resulted in a 19.7 – fold increase in the expected number of spores on a trap. Relative to 1m away, being 30m away decreased the expected number of spores by 13.5%, and being 75m away decreased the expectation by 76.5%. The effect of baiting a trap in model 2.2 resulted in an increase in the expected number of spores on a trap by a factor of 13.3.

Competition Experiment

Across all non-control plates, the number of counted *S. pensylvanicum* gametophytes was 444 whereas the number of *S. ampullaceum* gametophytes was 58.6% greater at 704. The best

candidate sub-model for predicting the species identity of a gamotophyte was the non-interactive base model including log spore concentration, proportion of input spores of *S. ampullaceum*, and region of the plate plus an interaction between the log spore concentration and the region of the plate and the 3rd order interaction between all three predictors (Table 3; model 3.1). Negligibly less ($\Delta AICc = 1.9 * 10^{-6}$) parsimonious was the same model plus an additional interaction between log concentration and input *S. ampullaceum* proportion (Table 3; model 3.2), we discounted this model from serious consideration because it failed to improve AIC or Nagelkerke's pseudo-R^2.

A type 3 analysis of deviance test was performed to examine if effects were significant even with full knowledge of higher order interactions. All effects were significant at $\alpha = 0.05$ (Table 3). The strongest effect as measured by analysis of deviance (Table 4) was the natural logarithm of the concentration, followed by input proportion of *S. ampullaceum* and then the ternary interaction. Region and the binary interaction of region and concentration were less influential but still significant.

The effect of moving from the center towards the edge of each dung pat as concentration and input proportion of *S. ampullaceum* increased was to shift the odds toward finding *S. pensylvanicum* relative to *S. ampullaceum* in the ternary interaction confidence interval plot (Fig. 2, panel A). This can be observed from the narrowing of the gap in log odds between the two species between center and middle, and middle and edge. The binary interaction showed increasing tendency toward finding no gametophyte as the concentration increased and the observation region moved from the interior to the exterior (Fig. 2, panel B). The main effects (Fig. 2, panel C) of being in the middle of the plate was to marginally increase the odds of finding a gametophyte of either species, the main effect of being on the edge was to decrease the

odds of finding an *S. ampullaceum* gametophyte and increase the odds of finding an *S. pensylvanicum* gametophyte. The main effect of increasing the sowing concentration was to increase the odds of finding a gametophyte of either species, with *S. pensylvanicum* experiencing a greater benefit. The main effect of increasing the input proportion of *S. ampullaceum* was to increase the odds of finding an *S. ampullaceum* gametophyte and decrease the odds of finding an *S. pensylvanicum* gametophyte.

Discussion

The results of this study suggest that the coexistence of *S. ampullaceum* and *S. pensylvanicum* is more complex than a simple tradeoff between competition and dispersal. The competition experiment demonstrated that *S. ampullaceum* is the superior competitor on average across the range of sowing densities and competitor ratios with 58.6% more *S. ampullaceum* gametophytes sampled than *S. pensylvanicum*. Relative competitive ability appears to be mediated by the moisture content of moose dung, as evinced by the competitive benefit for *S. pensylvanicum* growing toward the fringes of plate. We provide additional evidence for the findings of Cameron and Wyatt (1986) that Splachnaceae appear to be substrate, as opposed to dispersal limited. All moose dung placed within 10m of Splachnaceae populations were colonized, suggesting that adequate numbers of spores are transferred to fresh dung at short distances for both species. The results of the two spore movement experiments show that all baited spore-traps trapped spores irrespective of which species of Splachnaceae was nearby, and that spores disperse at least 75m. The results from the spore transfer assay suggests that, within peatlands containing *S. ampullaceum* and *S. pensylvanicum,* the abundance and position of populations is dependent on the availability of fresh dung, which hinges on the defecating behavior of moose. This contrasts with the frequent observation that patch tracking bryophytes are dispersal limited at a local scale

(Snäll et al. 2005; Löbel and Rydin 2010). The difference between our findings and those of other patch tracking bryophytes likely lies in the unique dispersal mode of Splachnaceae relative to other bryophytes, as well as the scale of dispersal. By using flies as dispersal vectors, Splachnaceae can overcome the tendency for spores to accumulate near the parental plant. This dispersal mode carries the risk of failing to disperse in the event that the moss fails to attract a disperser, but ensures dispersed spores will reach suitable substrate with higher fidelity than anemophilous transfer. This work did not look at the probability of dispersal at distances greater than 75 m, but the aggregated spatial distribution of target dung pats increases the likelihood that target dung pats will be near a source population (mosses are on average approximately 30m from their nearest two neighbours in one well-colonized peatland; Hammill unpublished).

The growth experiments suggest that the competitive asymmetry between the two Splachnaceae examined varies by location on the substrate, which we believe to be a proxy for moisture. *Splachnum pensylvanicum* was found more frequently toward the edges of the plate, indicating a potential growth advantage at low moisture, this is corroborated by field observations from Dickson (unpublished) in which *S. pensylvanicum* tended to be found toward the edges of mixed populations. This potentially corroborates the findings of Marino (1991) in which he showed competitive hierarchies amongst Splachnaceae (unfortunately not including *S. pensylvanicum*) depend on moisture regime. The patterning of bryophytes along a moisture gradient has also been observed for other peatland bryophyte species (Li and Vitt 1995). The position within the plate was treated as a proxy for a moisture gradient from the center of the plate toward the edge as the dung was observed to dry more quickly and tended to have fewer gametophytes to retain moisture towards the edges. We had hypothesized from observing Splachnaceae in the field that, perhaps, *S. pensylvancum* was more tolerant of desiccation, this could explain the increased odds

of observing *S. pensylvanicum* toward the edges of the dung pat.

Sowing density also had a differential effect on the two mosses as *S. pensylvanicum* experienced a disproportionate benefit at high sowing concentrations. It is possible that at low sowing concentrations *S. ampullaceum* spores grow relatively faster, occupying the most ideal portions of the dung, pushing *S. pensylvanicum* toward the fringes. At high concentration however *S. pensylvanicum* may be able to establish footholds in more ideal spaces just due to stochastic differences in germination time, although follow up work is needed.

Both species experienced improved odds of covering each spot on the plate at high concentrations, irrespective of interactive effects, this is evidence that there may also be facilitative interactions occurring at high density. It is known that gametophytes help moss populations retain water (Zotz et al. 2000), this benefit is likely indiscriminately shared with competitors, such that the mosses compete for space but facilitate each other's growth at high density due to increased water retention at higher gametophyte densities. It's been argued that facilitation may be an under-appreciated driver of biodiversity (McIntire and Fajardo 2014) which could certainly be the case amongst Splachnaceae. There was also some gametophyte growth on control plates. This could either be via spore movement during watering, or more likely, the fragmenting of protonemal growth during watering. In both species, protonema take on a 3-dimentional almost rod-like structure that is easily fragmented. It is possible, that this ease of protonemal fragmentation is, itself, an adaption to facilitate the rapid colonization of dung. The species spore dispersal experiment failed to detect dispersal differences between *S. ampullaceum* and *S. pensylvanicum*. It is known from the work of Marino and Raguso (2016) that *S. ampullaceum* and *S. pensylvanicum* attract different (but overlapping) fly faunas, and our expectation was that spore dispersal would differ as a consequence. This could mean that the fly

species responsible for the faunal differences between the two species do not play a pivotal role in dispersal, and that the more abundant shared vector species perform the bulk of the spore transfer or that despite their different faunal associations, both species have similar dispersal capabilities, at least at the scales we explored. The spores of the two moss species are indistinguishable microscopically, and so the true source of spores is only assumed to be the nearest set of populations. As the distance experiment demonstrates, the distances separating the species treatments may have been insufficient to isolate the experiment from cross-contamination. Differences in dispersal potential between *S. ampullaceum*, *S. pensylvanicum*, and mixed populations remains an open question, however, we do know that at the spatial scales we examined, spores of both species are dispersed to fresh dung. Nonetheless, there were clear signs of distance-dependence in the distance experiment (with weaker evidence from the species experiment) suggesting that the spores arriving at a dung pat depend on the distance to the nearest population, even at intra-peatland scales. It must be acknowledged that the sample sizes for these experiments were too small to make definitive conclusions about the dispersal patterns of the two mosses, but highlight avenues for future confirmatory experiments.

Through this work we've added weight to the observation that Splachnaceae mosses tend to be substrate, as opposed to dispersal limited as suggested by Wyatt and Cameron (1986) and that competition in mixed populations is generally dominated by *S. ampullaceum*, but is dependent on microsite differences that we've attributed to a mositure gradient. It has already been suggested by Hammill and Marino (2016) that these mosses may temporally segregate access to dung pats to minimize the influence of competition; however competitive asymmetry in that model was considerably more pronounced than observed in this experiment. We have preliminary evidence to suggest that conspecific Splachnaceae may experience varying modes of

ecological interaction depending on the niche axis of interest, likely being highly competitive for space, but potentially facilitative for water at high sowing density. This mixed mode of interaction needs to be considered seriously when examining bryophyte ecology as it may transcend the Splachnaceae. From the above work it seems highly likely that Splachnaceae in Newfoundland peatlands are coexisting through a mixture of temporal resource partitioning and asymmetric growth responses to moisture.

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Figures

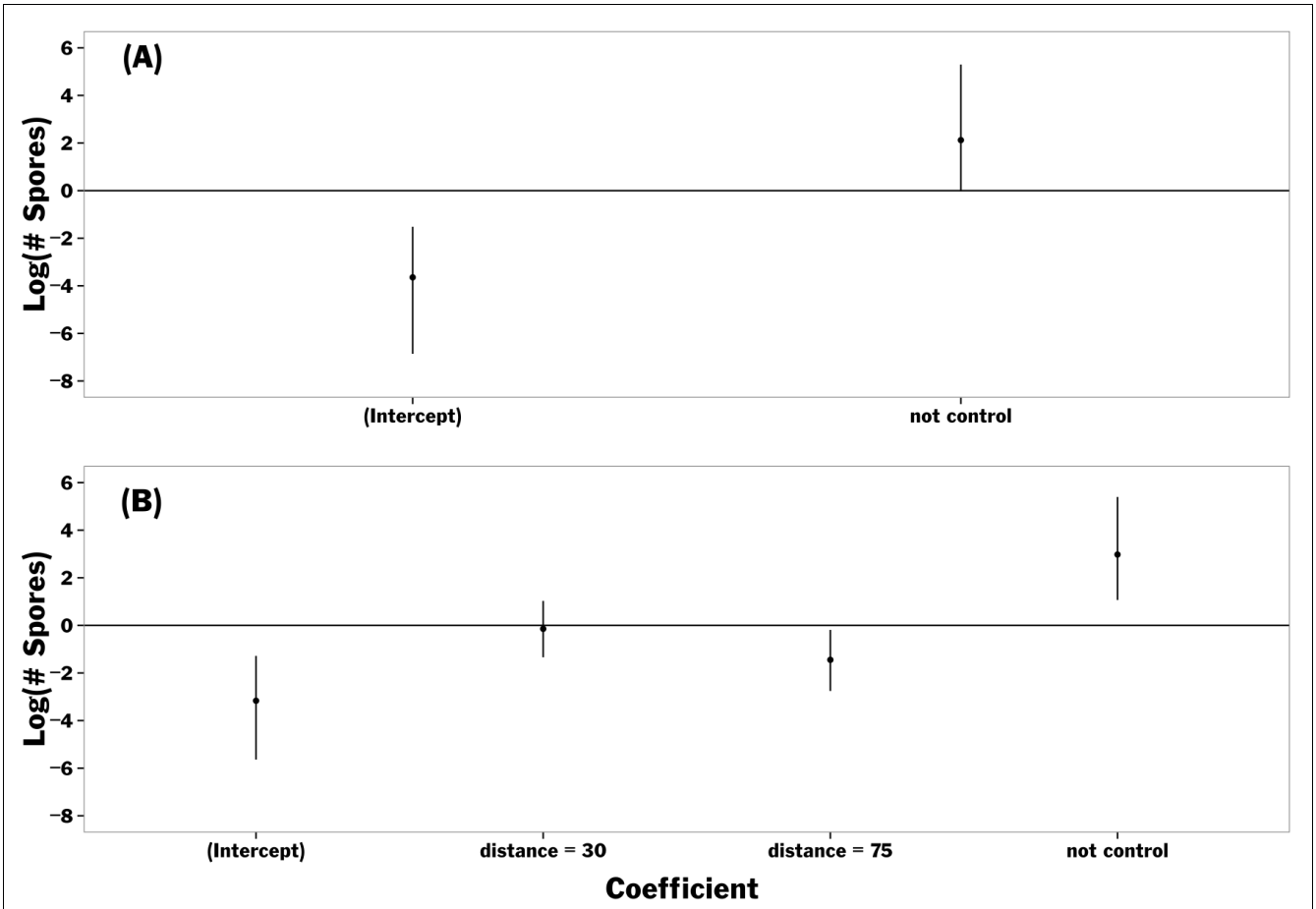

Fig 1. Coefficient estimates and 95% confidence intervals from the most best model (as chosen by AICc) in spore transfer experiments 1 (model 1.1; panel A) and 2 (model 2.1; panel B). Coefficients are the multiplicative change in the natural logarithm of the number of spores found on a given quadrant of a spore trap. The zero line represents no effect.

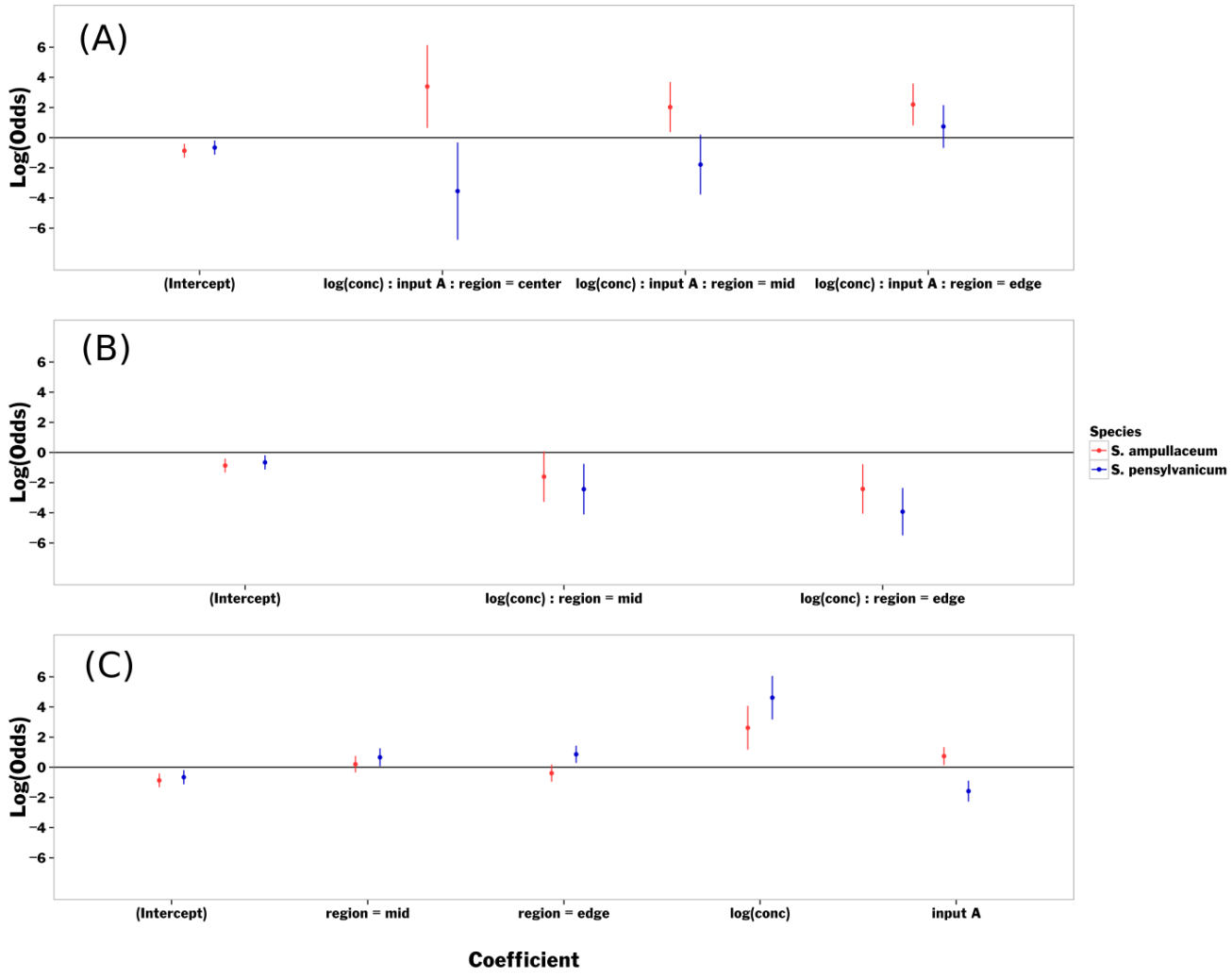

Fig 2. Coefficient estimates and 95% confidence intervals for coefficients in model 3.1 as the natural log of the odds ratio of finding either species relative to finding no moss at all on competition growth plates. Panel (A) shows ternary interaction effects, Panel (B) shows binary interaction effects, and Panel (C) shows main effects. The zero line represents no effect of the coefficient, with higher log odds representing increased chances of finding the species denoted by the colour coding.

Table 1a: Summary of model selection output for predicting spore movement in the species effect experiment. The formulae given are presented in the style of *lme4* and indicate the included predictors, with random intercept random effects are indicated by (1 | predictor). All predictors can be found in Table 2. K is the number of parameters. Log likelihood is the logarithm of the likelihood of each model given the data. AICc is the small sample size corrected Akaike's An Information Criterion, ΔAICc represents the difference in AICc between the given model and the most parsimonious model. Akaike Weights (ωAICc) represent the degree of support for a given model relative to the entirety of the set, evidence ratios are the degree of evidence for a given model relative to the most parsimonious model. Models are ordered by increasing AICc (decreasing support). Marginal R2 is the proportion of variance explained by the fixed effects of the model, conditional R2 is the proportion of variance explained by both the fixed and random effects of the model.

Model	Formula	K	Log Likelihood	ΔAICc	ωAICc	Evidence Ratio	Conditional R^2	Marginal R^2
1.1	C ~ B + (1 | G) + (1 | T)	4	-190.7	-	0.401	-	0.503	0.091
1.2	C ~ D + B + (1 | G) + (1 | T)	5	-190.2	0.924	0.253	0.63	0.507	0.1
1.3	C ~ (1 | G) + (1 | T)	3	-192.6	1.697	0.172	0.428	0.472	0
1.4	C ~ D + (1 | G) + (1 | T)	4	-192.4	3.299	0.077	0.192	0.476	0.006

1.5	C ~ S + B + (1 | G) + (1 | T)	6	-190.6	3.967	0.055	0.138	0.505	0.092
1.6	C ~ D + S + B + (1 | G) + (1 | T)	7	-190.1	4.986	0.033	0.083	0.508	0.101
1.7	C ~ D + S + (1 | G) + (1 | T)	6	-192.4	7.424	0.01	0.024	0.476	0.006

Table 1b: Summary of model selection criteria for predicting spore movement in the distance effect experiment.*

Model	Formula*	K	Log Likelihood	ΔAICc	$\overset{\omega}{\text{AICc}}$	Evidence Ratio	Conditional R^2	Marginal R^2
2.1	C ~ D + B + (1 | G) + (1 | T)	6	-158.3	-	0.629	-	0.697	0.256
2.2	C ~ B + (1 | G) + (1 | T)	4	-161.1	1.335	0.323	0.513	0.7	0.19
2.3	C ~ (1 | G) + (1 | T)	3	-164.5	6.024	0.031	0.049	0.656	0
2.4	C ~ D + (1 | G) + (1 | T)	5	-163.0	7.193	0.017	0.027	0.66	0.065

*Formulae are presented in the style of specification for lme4, with random intercept random effects are indicated by (1 | factor). The models in table 1a predict spore count on a trap (C) given fixed factors: B (trap baited or not), D (distance to nearest population), S (single- and mixed-species populations) and random factors: G (sampling group) and T (individual trap). In table 1b, the models predict spore count on a trap (C) given fixed factors: B (trap baited or not) and D (distance to nearest population) and random factors: G (sampling group) and T (individual trap).

Table 2: Predictors used for model Selection by experiment. Experiment indicates which set of models includes each predictor, symbol is the shorthand used in formulae. Type is whether predictors were fixed or random, and scale is the coding of each predictor.

Experiment	**Symbol**	**Predictors**	**Type**	**Scale**
Dispersal 1	D	Distance	Fixed	Binary {1m, 15m}
	S	Species (nearest population)	Fixed	Factor {*S. ampullaceum*, *S. pensylvanicum*, Mixed}
Dispersal 2	D	Distance	Fixed	Scalar {1-75 m}
Dispersal 1 and 2	B	Dung bait present?	Fixed	Binary {true, false}
	G	Sampling group (week)	Fixed	Factor {1,2,3,4,5}
	T	Tray	Fixed	Factor {1,2,3,4,5,6,7}
Competition	C	Spore Concentration	Fixed	Scalar {10,000 – 1,000,000 spores/mL}
	A	Proportion *S. ampullaceum* spores	Fixed	Proportion {0 - 1}
	R	Region of plate	Fixed	Factor {edge, middle, center}

Table 3: Total gametophyte counts for the competition experiment. Table shows the counts for *S. ampullaceum, S. pensylvanicum,* and uncolonized regions of the dung. Twelve regions were counted per plate along three transects

		Plate Region		
	Species	center	mid	edge
Sown	*S. ampullaceum*	297	245	162
	S. pensylvanicum	129	144	171
	Uncolonized	78	115	171
Control	*S. ampullaceum*	9	13	4
	S. pensylvanicum	5	13	24
	Uncolonized	22	10	8

Table 4: Summary of model selection criteria for predicting species identity on competition plates. The formulae given are presented in the style of linear modelling in R. Descriptions of each predictor can be found in Table 2. K is the number of parameters. Log likelihood is the logarithm of the likelihood of each model given the data. AICc is the small sample size corrected Akaike's An Information Criterion, ΔAICc represents the difference in AICc between the given model and the most parsimonious model. Akaike Weights (ωAICc) represent the degree of support for a given model relative to the entirety of the set, evidence ratios are the degree of evidence for a given model relative to the most parsimonious model. Models are ordered by increasing AICc (decreasing support). Nagelkerke's R^2 is an estimation of the proportion of variability in the data explained by the model.

Model	Formula*	K	Log Likelihood	ΔAICc	ωAICc	Evidence Ratio	Nagelkerke R^2
3.1	S ~ C + A + R + C:R + C:R:A	20	-1347.5	0	0.37	1	0.425
3.2	S ~ C + A + R + C:R + C:A + C:R:A	20	-1347.5	$1.9 * 10^{-6}$	0.37	1	0.425
3.3	S ~ C + A + R + A:R + C:R + C:A	20	-1348.4	1.9	0.14	0.38	0.424
3.4	S ~ C + A + R + A:R + C:R + C:R:A	24	-1345.3	3.9	0.054	0.14	0.427
3.5	S ~ C + A + R + A:R + C:R + C:A + C:R:A	24	-1345.3	3.9	0.054	0.14	0.427

[*]Formulae are given in the format of linear modelling in R with interactive effects denoted by the contributing predictor separated by a colon. S represents species identity, C represents the natural logarithm of the spore sowing concentration, A represents the input proportion of *S. ampullaceum* spores, and R represents the region of the plate (edge, mid, or center).

Table 5: Type III analysis of deviance table for the top competition model(3.1) as chosen by corrected AIC[*].

LR	Chisq	Df	Pr(>Chisq)
logConc	66.74	2	$3.21 * 10^{-15}$
inputA	43.09	2	$4.39 * 10^{-10}$
region	19.24	4	$7.06 * 10^{-4}$
logConc:region	30.57	4	$3.75 * 10^{-6}$
logConc:inputA:region	58.03	6	$1.13 * 10^{-10}$

Chapter 4: Grand Conclusion

For this study I set out to better understand how Splachnaceae mosses coexist. After individual-based simulation modelling and field experiments I think I now have a better grasp on how *Splachnum ampullaceum* and *S. pensylvanicum* coexist in Newfoundland peatlands. At the outset it seemed likely that there was a competition-colonization trade-off (Tilman 1982) and aggregation mediated coexistence (Marino 1988; Marino 1991) at play. My work suggests that *Splachnum ampullaceum* and *S. pensylvanicum* likely coexist by dividing dung resources temporally, as shown by the simulation model, and along a moisture gradient, as shown by the growth experiment. This partitioning allows the weaker competitor, in most cases *S. pensylvanicum*, to find an establishment niche. There are likely additional mechanisms that allow the two species to coexist; the spore dispersal experiment wasn't sophisticated enough to identify species-specific differences in relative dispersal ability, and hence we can't discount a competition colonization trade-off. However, even if additional mechanisms are at play, the simulation model suggests they probably aren't fully necessary for coexistence.

It seems that coexistence is more complex than I had anticipated. Perhaps the reason neutral theory is often so predictive is that there are many niche axes, and many determinants of vital rates, so finding species under the purview of the competitive exclusion principle is challenging (Hubbell 2001; Chave 2004). It also seems likely to me that we tend to have a selection bias for experimenting on species that are known to occur together. This would mean we are more likely to experiment on species that have a robust suite of stabilizing mechanisms, *sensu* Chesson (2000), at play, and so coexistence should be the expectation, not the exception. Coexistence seems to come down to what are two coexisting species doing just differently enough to avoid limiting each-other, although this is certainly not an original thought (Chesson 2000).

I think the next steps for consolidating our understanding of Splachnaceae ecology is to consider the role that differing vector fauna play in species interaction. Marino and Raguso (2016) have reviewed the similarities between Splachnaceae-fly networks and plant-pollinator networks, I think it would be fruitful to examine a spore dispersal network model. One could simulate a peatland as an undirected weighted graph, with each node having an identity as a moss population or dung pat with corresponding attributes. The edge weights would be related to distance. Then random draws from the fly distribution could be taken and allowed to wander the graph according to each fly species' signal preferences, as experimentally determined by Marino and Raguso (2016). This graph wandering process could be modelled as a first-order Markov process and so the long-term average time spent at each node for each fly species could be assessed. The network could evolve by adding new dung nodes, senescing moss nodes, and allowing dung nodes to convert to moss nodes.

There is also much to be learned by comparing Splachnaceae oviposition site mimicry with the mimicry used by angiosperms. We could integrate the odour profiles of Splachnaceae with those of angiosperm oviposition site mimics (Jürgens *et al*. 2013), this could yield interesting insight into the mimicry strategy used by these mosses.

For the last portion of this thesis I'd like to take the opportunity to give my thoughts on coexistence and ecology. Many of these thoughts fall outside the direct scope of my work but I think I'd be remiss in leaving them unsaid. I think we need to be moving in the direction of more individual-based modelling for ecosystems (DeAngelis and Mooij 2005). Though there is much to be gained from ground-up mathematical modelling, parsimony is just one optimizable quantity when trying to understand nature. Modelling individuals is becoming a more tractable task. Then simulating many organisms from those distributions becomes feasible, and we can create *in*

silico representations of entire ecosystems. Having accurate *in silico* representation would allow us to bootstrap the effort we put in to experimental work. Provided we can continually validate our models, orders of magnitude more experiments can be run on a computer than in the field. This is particularly true as computing gets faster and cheaper, there is much to be gained from embracing, at least in part, the big data paradigm. The advances in machine learning continue to march on every day, deep neural networks can now outperform humans on many tasks, like image recognition (Schmidhuber 2015; LeCun *et al.* 2015). These models can help us to filter what is and is not important from terabytes of simulation data (Gedeon 1997), allowing us to focus on promising avenues for research.

I think the complexity of nature may be too vast for us to comprehend on our own; there are so many models, mechanisms, and heuristics for ecology, it may no longer be possible for a single scientist or group of scientists to comprehend them all. This is why I advocate working on our levers. The more we can out-source our understanding of nature to central repositories and use machine learning and *in silico* experimentation to make inference, the more we can behave as meta-scientists, combining the learning of millions of researchers world-wide. I think we need to forward the agenda of teaching statistical, mathematical, and computational biology to ecology students earlier in their academic careers, so that ecology can be better positioned to capitalize on emerging data science tools and techniques.

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Appendix 1: Dispersal and Phenology

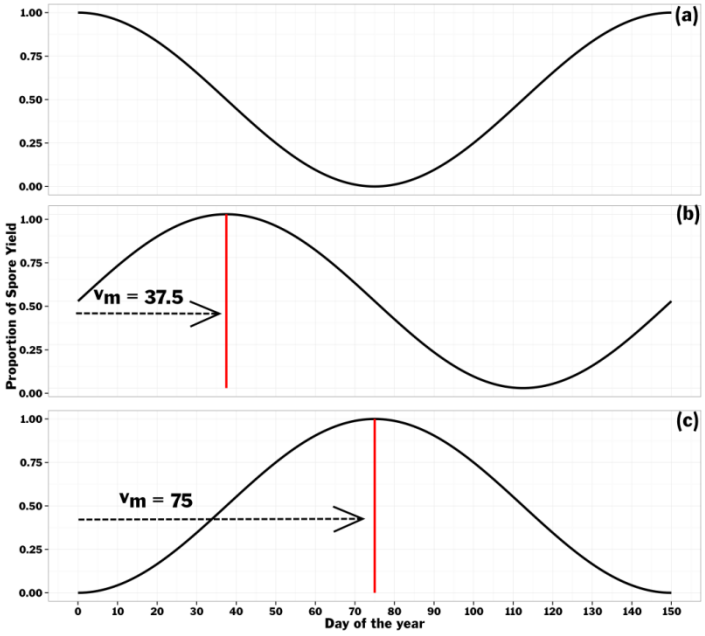

Fig. S1: Illustration of the effect of horizontal translation on the timing and magnitude of spore production. (a) shows the untranslated function, (b) shows a 37.5 day (sine) translation, (c) shows a 75 (negative cosine) translation.

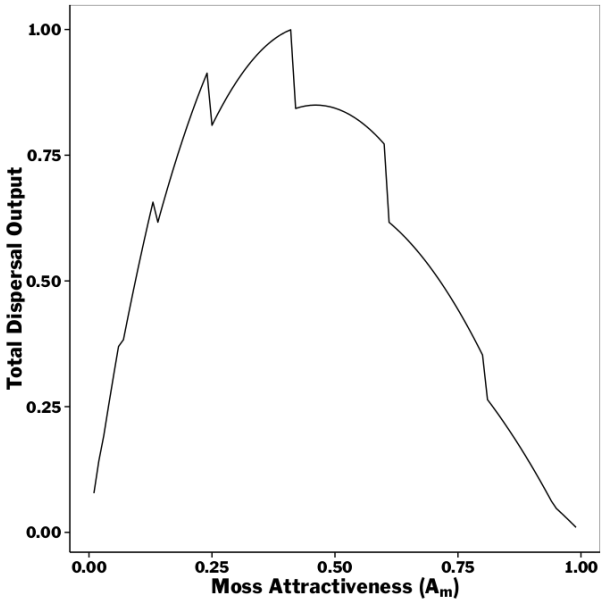

Fig S2. Total moss spore output to a given dung pat given moss relative attractiveness

Appendix 2: Understanding Variable Importances

Neural networks represent a complete computation system: any type of relationship, regardless of functional form, can be learned by a neural network given adequate architecture (Nielsen 2015). This differs from random forests, which are composed of an ensemble of weak learners (regressions trees) that are capable of learning non-linear relationships only through segmenting parameter space with respect to the predictors and fitting linear relationships within those subspaces. The forest itself performs an extra layer of non-linear learning via the aggregation of predictions with a voting system (Breiman 2001). This means that the importance of a predictor from the perspective of the random forest is more similar to how a human would weight the importance of that predictor, importance here being akin to variance explained (Louppe *et al.* 2013). In essence, the kinds of relationships learned by a random forest are much more similar to the kinds of relationships one might learn from data via conventional statistics, whereas a neural network will learn relationships more complex than would be discovered through traditional means. When interpreting the importance metric outputs of these two model types it is important to bear in mind that the simpler models built by the random forest algorithm will be more useful for human intelligible model building and can largely be taken as presented; this does not mean the neural networks importances are less valuable, but the interpretation needs to be performed with more caution. An additional advantage offered by the random forest is that a different set of randomly selected predictors is used by each tree and will frequently not include mutually informative predictors, this means that the importance metrics offered by a random forest can be used for feature selection (due to reduced codependence of predictors) with more ease than with the neural network importances (which depend critically on the presence of all predictors).

Appendix 3: Experimental Layout

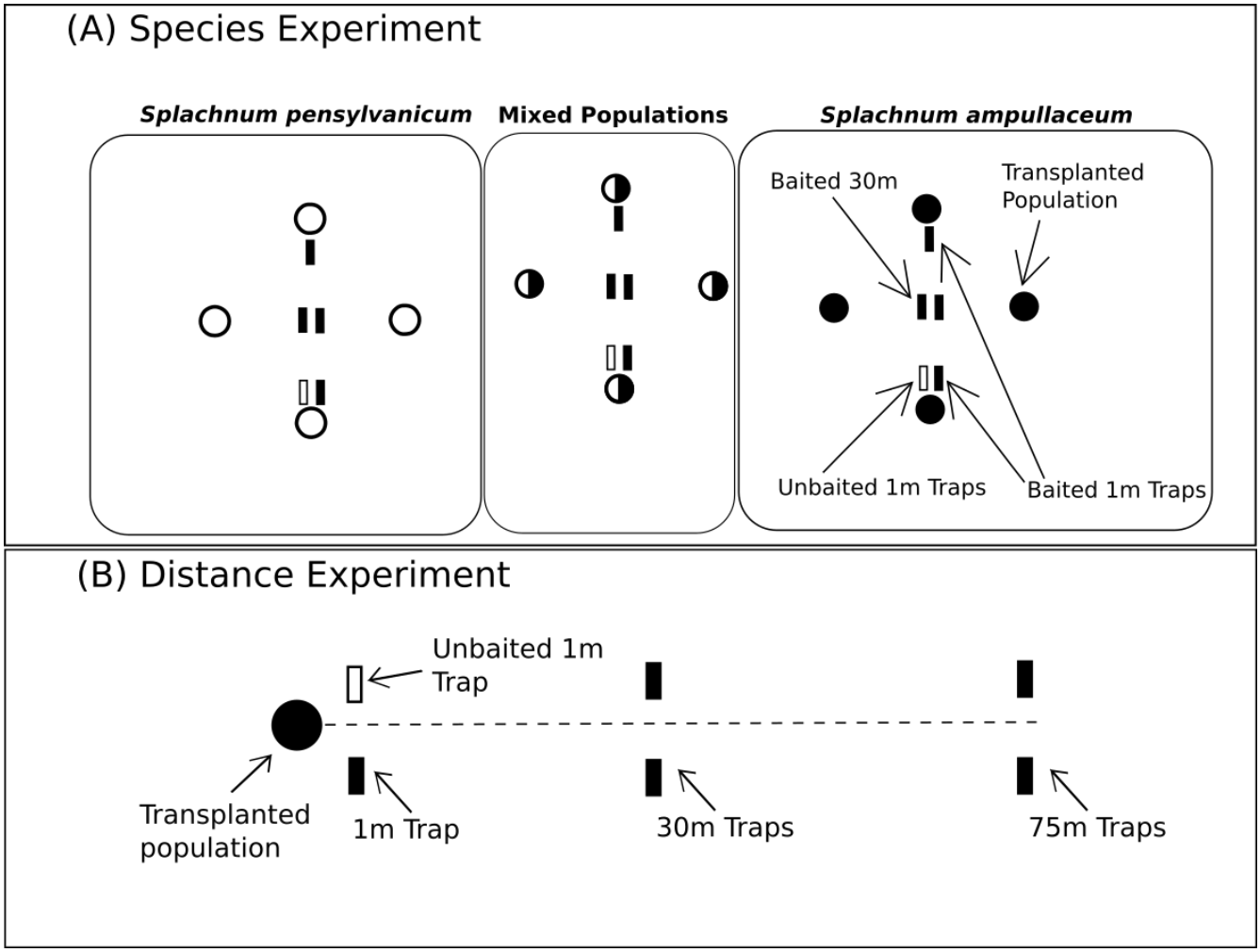

Fig S3. Experimental layout: Panel A shows the layout for the species experiment
Panel B shows the layout for the distance experiment.